# LABORATORY DIRECTIONS

IN

# GENERAL ZOOLOGY

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*By*

WINTERTON C. CURTIS

PROFESSOR OF ZOOLOGY, UNIVERSITY OF MISSOURI

SEPTEMBER, 1913

FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE
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# PREFACE

These notes are the outcome of one mimeographed and two printed editions, which have been written for the introductory course in zoology at the University of Missouri. The effort has been made with each revision to better adapt them to the needs of our students and to make corrections where the plan of work or the phraseology had proved unsatisfactory. As will be evident to any one with experience in work of this character, they are unusually full and this will doubtless be a matter of criticism by those who believe that the student should receive as little help as possible in the simpler parts of his laboratory study. The author agrees entirely with this contention and it is the very thing he has attempted in the writing of these laboratory directions and in the whole organization of his work in the teaching of general zoology. With this end in view, the work of the course is so planned that some laboratory work shall precede any text-book or lecture study upon a given form. Where the student begins his study of each animal with laboratory observation, it will be found that too brief directions result in his being constantly in need of help from the instructor. In such a plan of work he needs notes which will offer adequate direction and at the same time present a carefully studied attempt to make him determine for himself all the points which experience shows are made out by the better students. If, in addition, the instructors in a laboratory will try never to answer any question the student can be fairly expected to answer for himself, confining their attention to setting him right when he is on the wrong track with his material or has misunderstood the directions, and will, on the other hand, spend most of their time in explaining the broader aspects of the facts the student sees and in responding quickly to the kind of questions he asks when doing understandingly the work called for, the author believes that the

maximum of efficiency in laboratory instruction will be obtained, and these notes have gradually developed in conformity with this method of instruction. This procedure should, of course, not be pushed to the extreme of having all the laboratory work come first, because the student should have another chance at the material after his lecture and text-book study have given him a broader appreciation of what he can see in the laboratory, the ideal plan being to have the latter part of the laboratory study follow the main portion of the lecture and text-book assignments. When this plan is adopted, it has been our experience at the University of Missouri that students can be trained to work independently with the directions here given, while the advantage for lecture and text-book work lies in the fact that the student understands much more readily what he reads or hears after he has seen something of it at first hand. Thus a good deal of time may be saved, particularly in lectures, which, when they precede any first hand knowledge, often labor to explain what could be much better seen and mastered by the student without any preliminaries.

Most of the work has been written **de novo** for this course although the author has made no attempt at originality in many of the minor points, which have been accumulated from many different sources and sifted out until they represent the result of his teaching experience. In the original mimeographed edition, the notes on a number of forms were largely a modification of the Laboratory Directions in General Zoology by Professor E. A. Andrews of the Johns Hopkins University, to whom more than to any other one man the author feels indebted for his present habits of thought as a laboratory teacher. Very little now remains which would connect what is here given with the notes by Professor Andrews. In the section on the Earthworm, however, there are a number of paragraphs which have survived and perhaps a sentence here and there in the notes on the Frog, the Unicellular Forms and the Hydra. For permission to use these as they stand in the present edition, which is copyrighted and placed on general sale, I am indebted to Professor Andrews. The greater part of the notes were, however, first written and have since been revised

directly from the material and may be regarded as an outcome of the laboratory teaching of General Zoology at the University of Missouri during the past ten years. I have to thank my colleagues, George Lefevre, G. W. Tannreuther, and G. S. Dodds, and our assistants who, from year to year, have helped with each revision to eliminate the most obvious defects in phraseology and method.

WINTERTON C. CURTIS.

January 1, 1912.

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## PREFACE TO THE PRESENT EDITION

The present edition has been revised in the light of further experience at the University of Missouri and of suggestions from other institutions in which the directions have been used. Some additions have been made with a view to making the work more adaptable, particularly in courses where the plan for introductory work in zoology includes a somewhat wider range of forms. In this connection, the scope of the lecture and text-book work in General Zoology as given at the University of Missouri may well be stated. The course is one for five hours credit during one semester. The schedule calls for two lecture periods of one hour and three laboratory periods of two hours each, with reasonable latitude for the individual instructor in the substitution of quiz or lecture work for some of the laboratory periods. The plan of the laboratory work in relation to the entire course of study will be understood by the following outline:

I. Representative Forms of Animal Life.
   (Studied with reference to their structure, physiological processes and life-cycles and as living animals rather than as representatives of a particular phylum).
   (a) The Frog, as a familiar form.
   (b) Unicellular Forms, to illustrate the basic phenomena of living things.
   (c) The Hydra, as a simple metazoon.

(d) The Earthworm, Crayfish or Mussel, as a complex metazoon of distinctly different organization from the more familiar vertebrates. Only one of these is usually studied in full as outlined.

II. The Ontogenetic Development of Animals.
   (a) Cell Division, Maturation, etc.
   (b) Cleavage and Formation of the Germ-layers.
   (c) Life-cycle of Amphibia.
   (d) Embryology of Frog and Chick.

III. Ecology, Behavior, Intelligence, etc.
   (a) The Insects.
   (b) The Parasitic Worms.

IV. The Classification of Animals and their Phylogenetic Development.
   (a) Brief Examination of the Principal Phyla.

V. Organic Evolution, lectures and demonstrations only.

These five main topics, of course, receive emphasis throughout the work, but they are more extensively developed in connection with the laboratory matter above indicated. The course, therefore, deals with a rather limited list of animals, and while the student is given an opportunity to form some conception of the more important phyla there is no intention of taking him from end to end of the animal kingdom as is sometimes done in courses entitled General Zoology. The aim is rather to present the fundamental phenomena of living matter as illustrated by representative forms of animal life.

At the request of Professor W. M. Smallwood of Syracuse University, notes are added upon the bony fish and the snail. These have been placed in an appendix as they do not logically belong at any place in the scheme of the course as here outlined. For these notes and for the outline of work upon the arteries of the frog, I have to thank Professor Smallwood and the staff at Syracuse University.

WINTERTON C. CURTIS.

University of Missouri,
September 1, 1913.

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## INTRODUCTORY NOTE FOR THE STUDENT

Since the proper understanding of any topic studied in the laboratory often involves an exact knowledge of the facts previously ascertained in lectures, laboratory and text-book, the student should make his laboratory work a matter of thoughtful study and should attempt to correlate each new fact with what he already knows rather than allow this work to degenerate into an unthinking manipulation of hands and instruments. Drawings are used solely as a means of enforcing exact observation and recording the results of the same. They are without value for zoology save as they show evidence of an understanding of the subject matter. While the ideal scientific drawing is not without artistic merit, this is a subordinate matter when compared with accuracy of detail. A figure by an unskilled draftsman often shows more real understanding of the structures involved than one by a person who is merely clever with his hands. Neatness is an important item because, it tends to enforce the habits of careful work which are conducive to the best observation and representation.

In beginning laboratory work, the student should be guided by the following suggestions: Bring to your seat at the beginning of the period all the instruments and other articles likely to be used, thus avoiding unnecessary moving about the room. Follow the laboratory directions explicitly, asking for information only when you do not understand or do not find the material. Return promptly all slides or specimens not intended for your exclusive use. Handle museum and demonstration specimens with great care and do not take from the demonstration table, without permission, material intended for general use.

**Your co-operation is asked in the attempt to make the laboratory a quiet and pleasant place of work and in so handling its equipment that every one may derive the maximum benefit therefrom.**

## THE LEOPARD FROG (RANA PIPIENS)

PHYLUM, CHORDATA. SUB-PHYLUM, VERTEBRATA.
CLASS, AMPHIBIA.

### I. EXTERNAL FEATURES

(a) Examine a freshly killed specimen in a dissecting pan and note the general **shape** and **coloration** of the body, the soft slimy **skin, mouth, anus, nostrils** and **eyes.** What is the relative development of the **eye-lids** and of the **nictitating membrane,** a thin fold which can be drawn over the eye? Is there an iris and pupil as in your own eye? How does the ear-drum or **tympanic membrane** differ in position from the structure of the same name in your own body?

(b) Watch live frogs in the laboratory, or better in a pond, noting the **modes of locomotion** on land and water and any characteristic behavior. In a live frog under a bell-jar watch the **respiratory movements** of nostrils, throat and body and try to explain how air is forced in and out of the lungs. How is the squatting posture adapted for a quick escape from danger? If possible watch frogs in the awkward sprawled out position assumed when **floating** near the surface of the water and determine whether this is also a good position for a quick escape. Note the **hump** in the back and, by reference to a skeleton, the bones which cause this. Study skeletons of frog and man and compare, particularly the **skull, vertebrae, sternum, clavicles, scapulas, pelvis,** and **bones of the limbs.** Understand the terms, **dorsal, ventral, anterior** and **posterior, cephalic** and **caudal.**

**Exercise I.** Place the animal in the pan dorsal side up, pinning out the fore and hind limbs with digits spread apart. Show the specimen thus prepared to one of the instructors to make sure it is properly arranged, or compare with the one thus

pinned out at the centre-table. Have pencils carefully sharpened. Rule a faint line lengthwise in the middle of a sheet of drawing paper and, using this to represent the median plane of the animal, show the strict bilateral symmetry which the outside exhibits. Draw on a scale of 1 as thus seen from the dorsal aspect. Measure all the distances accurately with ruler or compasses and show all the parts to which your attention has been called in the foregoing section so far as they appear in this view. Finish the drawing in simple outlines, without shading, as in the figures posted to illustrate this method of drawing. Label thoroughly, making index lines with a ruler and arranging the lines and the names to which they lead neatly.

## II. THE MOUTH CAVITY

(a) Open the mouth and feel the bones in the upper and lower jaws. How are the teeth distributed? Fine the **vomerine teeth** near the **internal nostrils.** Explore the nasal passages with a guarded bristle. How do they differ from those of man? Posteriorly the mouth narrows down and passes into the **oesophagus,** the diameter of which may be determined by probing with the handle of a needle. Press down the eyes from without and observe what happens. The frogs thus "pull in their eyes" when hiding beneath stones or other objects on the bottom. What is the shape and attachment of the **tongue?** On the mid-line of the floor and well back toward the oesophagus is an oval area with a slit in the middle, the **glottis,** above and on each side a large pit, the **Eustachian recess** or **tube.** Compare these features with what you know of the human body. In a male frog there are two small holes near the angles of the jaws leading into right and left cavities known as the **buccal sacs.** These may be demonstrated by inflation with a blow-pipe.

**Exercise 2.** Understand these features thoroughly and be prepared to show the relative position of the several parts by drawing a sagittal section of the head, or making surface views of the roof and floor of the mouth cavity.

## III. THE COELOMIC CAVITY

(a) Place the specimen in a pan ventral side up and fasten with pins through the limbs. Pick up the skin along the ventral mid-line with forceps and **cut through the skin only,** leaving intact the heavier layer of muscles which is separated from the skin by an open space. Continue the cut as a median longitudinal incision extending from the jaw to the cleft between the hind legs. Cut out at right angles to this in the region of the fore limbs and again at the posterior termination of the cleft. Reflect the flaps thus formed and pin out in this position. The extensive space between skin and muscles is called a **lymph sinus.** Examine the thighs and fore limbs, making small cuts through the skin only and exploring with a bristle the cavities thus disclosed. Can you find how these lymph cavities are separated from one another? Note fine branches of blood vessels extending over the skin and large right and left vessels which lie along the partition separating the ventral abdominal lymph sinus from those on the dorso-lateral surfaces. These are the **great cutaneous veins.** The **cutaneous arteries** may be found close to them but are not so readily distinguishable since they are no longer distended with blood. What fact regarding the distribution of veins and arteries do you discover upon studying the finer branchings under a hand lens? Along the mid-line, in the layer of the muscles, is another blood vessel, the **anterior abdominal vein.**

(b) With forceps, lift up the muscle layer in the region of this median vein and cut into the body-cavity or **coelome,** being careful not to injure the underlying **viscera.** Cut along the mid-line as far forward as the **sternum** and then **have one of the instructors help you remove a portion of this bone.** Expose the organs dorsal to the sternum by stretching out and pinning the fore limbs. Cut out at right angles posteriorly and reflect the flaps as in the case of the skin.

(c) If the specimen has not been dead too long, the heart will be still beating. Without injuring the heart, open the sac-like **pericardium** in which it lies and note the **ventricle**

which is single and the **auricles** of which there are two. Leading out anteriorly from the ventricle is a large vessel, the **truncus arteriosus** which divides right and left. On lifting up the posterior end of the ventricle the **sinus venosus** may be seen leading into the right auricle.

**Exercise 3.** As in the case of the mouth cavity, be prepared to represent these points by a simple diagram.

(d) As the organs lie in place, one can at once recognize the **liver** with its lobes and the coils of the **intestine.** On the observer's right, the **stomach** comes out from beneath the liver and passes into the intestine as it bends sharply forward. Between the liver lobes is the **gall-bladder.** Lift up the liver and find the **pancreas,** lying in the angle made by the stomach and the beginning of the intestine. Find the dark red **spleen** among the coils of intestine and beneath the posterior end of the stomach. Also find where the **small intestine** enlarges into the **large intestine** and the anterior end of the stomach where it communicates with the oesophagus. Locate the **lungs** and inflate them through the glottis with a blow-pipe. On the dorsal face of the body wall, find the dark red **kidneys** with finger-shaped **fat bodies** at one end. In a male, the **testes** are a pair of bean-shaped organs attached to the ventral face of the kidney. In the female, the **ovaries** are attached in a similar manner, but are larger particularly in the fall and winter when the eggs are maturing for the spring breeding season. The **oviducts** are greatly convoluted tubes lateral to the **kidneys.** Rudimentary oviducts are often found in young males.

(e) **With the direction of an instructor,** remove the digestive organs from the body by severing the tract across the oesophagus and large intestine. Note in so doing the **mesenteries** and how the blood vessels run through them. The shiny membrane which covers these organs and lines the abdominal and pericardial cavities is the **peritoneum.** Understand from the lectures and text-book the meaning of the terms **visceral** and **somatic layers** of peritoneum, and how the mesenteries are related to the same.

(f) To study the removed digestive tract, begin at the posterior end and unravel the intestine by cutting the mesenteries which hold the various coils in place. Stop this process before the region of the pancreas is reached and begin to pin out in the dissecting dish under water, reflecting the liver lobes **as in the specimen placed on the center-table.** Find the **bile-duct** which enters the intestine about opposite the middle of the pancreas, through which it passes on its course from the liver and gall-bladder. The term **duodenum** is applied to the region of the intestine where the bile-duct enters.

**Exercise 4.** Draw, on a scale of 3, showing the various parts of the tract and its appended glands (pancreas and liver). Finish as a simple line drawing and label thoroughly with index lines, etc., as in Exercise 1.

(g) With a sharp scalpel, cut the stomach in two transversely. Examine the cut surfaces and make out the layers as follows: **peritoneum, muscle layers, sub-mucosa** and, lining the tract, the **mucous membrane.**

(h) Slit the tract lengthwise from rectum to stomach, wash out thoroughly and examine under water, noting the character of the lining in different regions. Look for the opening of the bile duct into the duodenum.

(i) Cut thin slices of the liver lobes and examine the cut surface under water.

**Exercise 5.** Make diagrams on a scale of 2 or 3 to illustrate these points (g), (h) and (i).

## IV. THE URINO-GENITAL SYSTEM

(a) **The Female Organs.** If the specimen is a female you have already identified the paired **oviducts and ovaries.** Examine the latter and note how each ovary is swung to the back of the body cavity by a membrane, similar to the mesentery of the gut, but called the **mesovarium.** Notice the spherical **eggs** of which the organ is composed. Remove both the ovaries by cutting along the mesovaria, being careful not to destroy the **kidneys** and **fat-bodies.** Follow one oviduct

to its anterior end and find where it opens into the body-cavity. Leaving the left oviduct intact, cut away the peritoneum which holds its coils in place and without breaking the duct itself get an idea of its total length. Identify the **urinary bladder** and the stump of the **large intestine** and, leaving these intact, find the place at its posterior end where each oviduct dilates into a thin-walled sac. Notice how this dilatation passes back dorsal to the rectum. The eggs become detached from the ovary during the breeding season and pass into the openings at the anterior ends of the oviducts. In their passage through the oviducts they receive their jelly-like membranes. Finally, they accumulate in the dilatations at the posterior ends of the oviducts in preparation for the act of laying.

(b) Examine now a skeleton and understand how the large intestine is placed with reference to the bones of the pelvis. **With the aid of an instructor,** cut through the pelvis along the mid-line, in doing which you must exercise great care not to injure the underlying parts. Spread the cleft apart and, within the region thus exposed, cut away the peritoneum at either lobe of the bladder, and by breaking away the connective tissue free the **neck** of the same down to its entrance into the **cloaca** or terminal region of the alimentary canal. Leave the bladder intact and picking up the stump of the large intestine distinguish clearly between this and the two dilatations of the oviducts. Dissect the dilatations away from the rectum down to their openings into the cloaca and, lifting each dilatation, find the underlying **ureters** or ducts from the kidneys. Follow these last to their union with the cloaca. Notice that the urine, feces and reproductive products are all passed into the terminal portion of the alimentary canal, which is therefore called the cloaca. Pass a bristle into the anus and by moving it about determine the diameter of this cloacal region.

(c) Note again the **fat-bodies** at the head of the kidneys, their shape and mode of attachment and the yellow stripe along either kidney which is the **ad-renal body.**

**Exercise 6.** Draw this system from a ventral view on a scale of 2 or 3, showing the above points and be prepared to make diagrammatic figures of dorsal and lateral aspects.

(d) Examine the organs of the other sex, as dissected in neighboring specimens, and compare with your dissection.

(e) **The Male Organs.** If the specimen is a male, note the light colored **testes** and the mesentery-like membrane, the **mesorchium,** which holds each in place against the **kidneys.** Look in the mesorchium for fine ducts, **vasa efferentia,** which convey the sperm from the testes to the kidneys. Note the **fat-bodies** and the **adrenal bodies** which appear as a yellow stripe on either kidney. Cut away the peritoneum which holds either lobe of the **urinary bladder** and, without destroying the bladder or large intestine, find the whitish **ureters** leading back from either kidney. Free the bladder from its attachment in the median posterior region, leaving its union with the cloaca intact.

(f) Examine a skeleton and understand how the large intestine is placed with reference to the bones of the pelvis. **With the aid of an instructor,** cut through the pelvis along the midline, in doing which care must be exercised not to injure the underlying parts. Spread the cleft apart and dissect away the connective tissue until you can see the **neck** by which the bladder opens into the **cloaca** or terminal region of the digestive tract. Lift up the large intestine and follow out in the same way the **ureters** or the ducts from the kidneys. Notice that the urine, feces and reproductive products are all passed into the terminal portion of the alimentary canal which is therefore called the cloaca. Pass a bristle into the anus and by moving it about determine the diameter of this cloacal region. In the frog the **seminal fluid** passes from the testes into tubules of the kidneys and along these until it reaches the ureters and so the cloaca and anus. In immature specimens, rudimentary structures comparable to the oviducts are often highly developed. Later, these degenerate and become the **seminal vesicles** in which the ripe spermatozoa are stored. These should be examined in chart figures if not seen in dissection.

**Exercise 7.** Draw the dissection, on a scale of 3, showing the bladder reflected to one side and the rectum to the other.

(g) Examine the organs of the other sex, as dissected in neighboring specimens, and compare with your dissection.

## V. THE DORSAL REGION OF THE BODY CAVITY

(a) **The Kidneys and Their Blood Vessels.** Before beginning this dissection, remove the heart and lungs in the following manner. With strong scissors make a transverse cut through the floor of the mouth about midway between the heart and the anterior end of the jaws. Lift up the posterior edge of this incision and cut backwards on either side dividing the oesophagus into dorsal and ventral halves. **Taking care not to injure the lungs,** you will thus remove a rectangular piece from the floor of the mouth and oesophagus with the heart and lungs attached and uninjured. Put this away for future study.

(b) With forceps, take hold of the peritoneum on the midline near the head of the kidneys and by gently raising it see the extent of the **sub-vertebral lymph sinus.** If not already opened by the removal of the oviducts, the cavity of the sinus should be further exposed by cutting the peritoneum along the lateral border. Lifting the kidney of one side, make out the large **renal portal vein** which runs along the outer margin of the kidneys, sending off branches, also the **dorsal aorta,** a large artery which lies behind the kidneys and is formed anteriorly by the union of two large **systemic arteries.** Note how and where the dorsal aorta gives off smaller arteries. One of these, larger than the rest, is located at the junction of the two systemics and is the **coeliaco-mesenteric** which supplies the greater part of the digestive tract.

(c) In following the directions given in this paragraph remove the kidneys and their veins, but **not the dorsal aorta.** Cut any of the small branches of the dorsal aorta which prevent the removal of the kidneys, **leaving their stumps long enough to be recognizable.** Cut the peritoneum where it remains attached around the anterior and lateral margins of the sinus and cut across the ureters and renal portals behind. This will entirely free the kidneys **and leave the dorsal aorta and the two systemics uninjured and in place.** Upon examination of the kidneys under water you can now see the single out-going vein, **post cava,** which lies between them on the ven-

tral side and the two incoming **renal portal veins** passing along the outer margin of either kidney also fine branches from the dorsal aorta.

**Exercise 8.** Draw the kidney and its blood vessels on a scale of 3, showing by arrows the direction of blood flow.

(d) Continuing with the parts not yet removed, examine the dorsal aorta posteriorly and determine the origin and distribution of the two **iliac arteries.** Lift the portion of the oesophagus remaining in the specimen and look for blood vessels which leave the systemics. What becomes of the systemics when followed anteriorly? Remove the roof of the oesophagus and mouth far enough forward to see two **carotid arteries** which pass on either side into a foramen of the skull. Do these come from the systemics?

(e) **The Spinal Nerves.** Note the segmented back-bone covered by a shiny connective-tissue. Examine a skeleton and see how the back-bone terminates in a peculiar bone the **urostyle.** Note three pairs of large white nerves on either side of the dorsal aorta. Close against the body wall and in front of these nerves are three smaller pairs which pass diagonally backwards. Working on one side only, lift up and trim away the muscles and connective tissue until you find one large and two small nerves anterior to those just mentioned. In this connection, notice the large **subclavian arteries** which originate from the systemics and pass out along the largest of the nerves. What part of the body do they supply? The foregoing nine pairs of **spinal nerves** are numbered I to IX from in front backwards. There is in addition a very small X nerve which will be seen presently. There are connections between nerves II and III, which constitute the **brachial plexus,** and the more complicated unions of nerves VII, VIII and IX which form the **sciatic plexus.**

(f) The **Sympathetic System** is not difficult to understand, although considerable care is necessary in demonstrating to one's own satisfaction the various points. To study it successfully the dorsal aorta and the two systemics must still remain intact. Begin by taking the dorsal aorta between your forceps

just where the two systemic arteries unite, lift gently and look for two delicate nerve cords running with a wavy outline in the connective-tissue a little to the side of either systemic artery. These are the two **main trunks** of the **sympathetic system.** Each consists of a series of connected ganglia and forms an unbroken nerve extending along **ventral** to the pairs of spinal nerves and parallel to the long axis of the body. Taking care not to pull too hard on the dorsal aorta, look carefully at the sympathetic nerve of one side and note that there are short transverse nerves, the **rami communicantes** (singular, ramus communicans), connecting it with the successive spinal nerves. These rami are most easily recognized in the case of the IV, V and VI nerves. In the anterior region, they are represented by the fusion of the sympathetic trunk with the ventral face of nerves I, II and III. Demonstrate this fusion to your satisfaction. Follow the sympathetic system posteriorly and, by careful lifting, make out the number of rami going to the nerves of the sciatic plexus. In this posterior region there will be noted some yellowish swellings of the cord. These are the **ganglia** of the system. How many can you find and how are they placed with reference to the rami communicantes Examine now the sympathetic cords in the region of spinal nerves I, II and III. The ganglia are here represented by swellings of the cords where they cross the spinal nerves.

(g) The ganglia which are opposite nerves IV, V and VI are too small for very satisfactory demonstration in gross dissection, but their position is readily ascertained since they lie at the points on the cord where the three conspicuous rami take their departure. We have therefore discovered a sympathetic ganglion and a ramus communicans for each spinal nerve thus far examined. A tenth and last ganglion is usually found connected with the X spinal nerve, but it is very difficult of demonstration and the number of ganglia in the posterior ends of the cords is often subject to considerable variation. When the aorta is carefully lifted, the stumps of fine nerves will perhaps be found here and there passing from the sympathetic trunks in a ventral direction, one that is largest and most conspicuous

passes along side of the coeliaco-mesenteric artery. This is the nerve which runs to the **solar plexus.** For the distribution of these branches of the sympathetic system see description in your text-book.

(h) Cut through one of the systemic arteries just above the union with its fellow and, reflecting it outward, examine the **periganglionic glands,** large whitish masses which surround the exit of the spinal nerves from the vertebral column. Is there one for each spinal nerve? Note just where each spinal nerve takes its origin. Find the X pair of spinal nerves and compare the same in your own and other specimens.

**Exercise 9.** Construct a diagrammatic drawing to show the spinal and sympathetic systems, on a scale of 3 or 4, as seen from the ventral or lateral aspect. The back-bone may be shown, but the dorsal aorta is better omitted.

## VI. JOINTS AND MUSCLES

(a) Examine a skeleton to see how the "**head**" of the femur fits into a depression, the **acetabulum,** of the pelvis; the whole forming a "**ball and socket**" joint. There is a less perfect one where the humerus articulates with the pectoral girdle. At the elbows and knees examine the nature of the articulating surfaces of "**hinge-joints.**" Your specimen will probably have one of the hip joints still intact and this should be examined by removing the muscles until the strong **capsular ligament,** which encloses the head of the femur, is found. How is this ligament attached to the femur and to the pelvis? Slit open the ligament and expose the cavity of the **synovial capsule.** In what directions can such a joint work?

(b) Strip off the skin of one hind limb and find the **gastrocnemius,** a large muscle constituting most of the "calf" of the leg. To what bones are the tendons of its proximal end attached? Distally it is continued into the **tendo Achillis.** Where is this in the human body? What movements do the contractions of the gastrocnemius produce? This may be determined by cutting the muscle in two at its middle and

pulling on the stumps. Other muscles, which produce movements of the knee and ankle joints, may be examined to understand further how motion is produced at a hing-joint.

(c) If the nerves of the sciatic plexus are followed outwards in the hind limb, their distribution to the muscles will be observed, particularly one which innervates the gastrocnemius. This muscle and nerve are often used for experimental purposes.

## VII. THE CENTRAL NERVOUS SYSTEM

(a) Pin the specimen down dorsal side up and remove the skin from anus to nostrils. Note in so doing the extensive lymph sinuses and the rich blood supply of the skin. Anteriorly between the eyes the roof of the skull will be seen. Examine a skeleton and see what bones you must cut through to expose the brain and spinal cord. It may now be found more convenient to cut off both hind limbs close to the trunk and to hold the specimen in the palm of the left hand as you remove the flesh. Use your scalpel to pare off the muscles until the spinal column is exposed, then scrape away the muscles on either side of this until you reach the transverse processes of the vertebrae. With the vertebral column thus exposed, pare down the tops of the vertebrae until the spinal cord is reached. **Being very careful not to cut into the cord and brain,** continue this paring process until you can see the entire cord and brain and by chipping away on the sides expose the full width of both. In doing this you will probably injure more or less the two membranes which surround the brain and cord. They are, the outer and thicker **dura mater,** and the inner and more delicate **pia mater.** The former lines the vertebral and cranial cavities, the latter is the pigmented membrane which closely invests the surface of the brain and cord and is in places distended with blood vessels. From this point on the dissection should be made under water.

(b) Beginning at the anterior end of the brain find the two large **olfactory nerves** passing forward to the nasal region. The **olfactory lobes,** from which these nerves originate, are not very

distinctly marked off from the **cerebral hemispheres** which constitute the greater bulk of this anterior region of the brain. Next is a narrowed portion, the **thalamencephalon,** followed by the paired **optic-lobes** and behind these, if it has not been destroyed, a dark area formed by a mass of blood vessels in the **choroid plexus.** Pull the choroid plexus free along one side and reflect it to show the **cerebellum,** a transverse ridge, and the **fourth ventricle,** a triangular cavity in the anterior end of the spinal cord. This anterior end of the cord, which increases in diameter before it merges into the brain, is the **medula oblongata.** If not pulled off with the pia mater, a delicate protuberance, the **pineal body,** may be seen on the mid-line at the anterior end of the thalamencephalon. By gently pressing the brain aside in the region of the thalamencephalon, find the **optic nerve** which leads from the ventral side of the brain to either eye. Another pair of large nerves, the **fifth cranial,** will be found coming off from the brain in the region beneath the optic lobes. There are a number of other cranial nerves but their dissection is very difficult and you are therefore referred to figures in your text-book where their position is shown.

(c) The ten pairs of spinal nerves, seen on the dorsal surface of the body cavity, arise from the spinal cord. The largest of them can now be made out leaving the cord by dorsal and ventral roots.

**Exercise 10.** Draw the brain and cord from a dorsal view on a scale of 3 showing the parts above made out. Indicate by an outline the position of the head, fore-limbs and eyes. If the exact outline of the cord is not clear, it may be determined after removal as in paragraph (d) below.

(d) Remove the cord and brain by lifting gently and cutting the nerves. Place in water and study the lateral and ventral aspects. Draw if time allows, comparing with figures or models of other vertebrate brains.

## VIII. THE LUNGS AND LARYNX

(a) Examine these organs from the specimen cut out and laid aside earlier in the work. Note again the character of the **glottis.** Find the **hyoid cartilage** in the floor of mouth. Locate the **larynx** and carefully clean off its ventral surface to see where the lungs connect with it. Is there a wind-pipe or **trachea** as in the human body? Separate the dissection into right and left halves, by a cut passing exactly along the mid-line and through the glottis. This is best done by a careful use of the scissors. The **laryngo-tracheal** chamber into which the glottis opens is now exposed. Find the **vocal cords** and the opening into either **lung.**

**Exercise 11.** Make a large outline of a frog's head and anterior part of body from a side view. Add to this an outline of the mouth cavity, the beginning of the oesophagus and the larynx and lung as they appear when cut as above. Specimens of whole frogs cut in this way may be examined at the centre-table.

## IX. PHYSIOLOGICAL EXPERIMENTS

(a) **Gastric Digestion.** Clean thoroughly three test-tubes and fill each one-half full of **distilled water.** Put into the first 3 or 4 drops of **hydrochloric acid** and a little **pepsin,** into the second the pepsin, but no acid, and leave the third with only the water. Put a small amount of some suitable proteid material into each test tube and watch the changes during the first few hours. Look up text-book and lecture notes on gastric digestion. Record the changes taking place in each tube.

(b) **Dialysis Experiments.** Make a simple dialyser by tying a piece of some animal membrane over the end of a wide piece of glass tubing. Fill about half full with a solution containing sugar in distilled water and place in a somewhat larger cylindrical vessel. Pour distilled water into the larger vessel until the liquid stands at the same level in the two. Set aside

and after an hour or two taste the water of the outer tube. Record the results.

(c) In place of the salt or sugar solution put some white of egg dissolved in water into the inner tube. After it has stood for about the same time as the other test the outside water for albumen. This may be done by pouring a little into a test tube and heating. Note results and then test in same way the fluid of the inner tube to which the solution of albumen was added.

(d) **Central Nervous System.** Follow the demonstrations with frogs in which (1) the cerebral hemispheres, (2) the entire brain and (3) the spinal cord have been successively destroyed and write out your conclusions regarding the functions of the various parts of the nervous system, also the importance of "intelligent" acts in the frog's daily existence.

(e) **Muscular Action.** Follow the demonstrations of the gastrocnemius muscle and its nerve and write out your conclusions regarding the nature of nervous control over muscle and the stimuli which affect muscle and nerve. After observing the action of the heart of a frog or turtle some time removed from the body, write out a statement of the meaning of **death** in the light of these facts.

## X. THE HEART AND BLOOD VESSELS

(a) The course of arteries and veins is more readily traced after these vessels have been injected with some colored mass. Specimens so prepared may be dissected for the blood vessels and at the same time as a review of the organs and systems already studied.

(b) **Heart.** In a specimen freshly anaesthetized, examine the uninjured **pericardium** and consider what important functions this may possess. Determine again the main divisions of the heart as in (c) on p. 11, and the exact order of their contraction. Refer to diagrams of the internal mechanism of valves, etc.

(c) **Arteries.** Leading away from the ventricle is the **truncus arteriosus** which divides right and left over the anterior margin of the auricles into two parts. Each of these again subdivides into three **aortic arches.** Of these three, the most anterior is the **carotid,** the middle the **systemic** and the most posterior the **pulmo-cutaneous arch.** The aortic arches subdivide as follows: The carotid arch soon divides into the **lingual** or **external carotid** and the **internal carotid,** the **carotid gland** occurring at the point of division. The pulmo-cutaneous arch divides almost immediately into the **pulmonary** and the **cutaneous.** The systemic arch passes dorsally and posteriorly, uniting with its fellow to form the **dorsal aorta,** as seen in the earlier dissection. Each systemic gives off an **oesophageal,** an **occipito-vertebral** and a **subclavian,** the last being known as the **brachial** after it reaches the arm and dividing at the elbow into the **radial** and **ulnar.** The dorsal aorta gives rise to: (1) the **coeliaco-mesenteric** which branches into the **coeliac,** to stomach and liver, and the **mesenteric,** to small intestine; (2) the **urino-genital arteries;** (3) the **lumbars;** (4) the **posterior mesenterics.** Posteriorly, the dorsal aorta divides to become the **common iliacs,** from which arise the **epigastrics** and **recto-vesicles.** In the thigh, the common iliac is known as the **sciatic** and gives off the **femoral.** At the knee the sciatic divides into the **peroneal** and **tibial.**

(d) **Capillaries.** The branches of the three aortic arches thus distribute the blood from the heart to all parts of the body and end in the network of capillaries. Examine a demonstration of the capillary circulation in the web of a frog's foot and determine the following points: Where does the pulse die out? What is the diameter of these smallest blood vessels as compared with the diameter of the corpuscles? How would you distinguish between arteries and veins? What is the structural basis for the interchange of material between the blood and the tissues?

(e) **Veins.** The dissection of the veins in the frog is of greater interest from the standpoint of comparative anatomy than along the general lines of physiology desirable in the

present study and may, therefore, be dispensed with in a course of this nature. Particularly as a considerable number of the larger veins have already been examined by dissection. These isolated portions should now be reviewed and put together by the examination of the present specimen and of text-book or chart diagrams. In this connection, consider the blood supplies of the kidneys and the liver and the meaning of the term "**portal system.**" Also the phenomenon of cutaneous respiration and how the oxygenated and unoxygenated blood occur in the veins and arteries. Again, can we speak of "pure" and "impure" blood without a further definition of terms?

## THE USE OF THE MICROSCOPE

### I. PARTS OF THE INSTRUMENT

(a) If you have previously used a microscope and are thoroughly familiar with its working, examine carefully the instrument assigned to you, noting the peculiarities, and then read over this section as a review. Special work will be assigned if you are ready to proceed before other members of the class who are using the instrument for the first time.

(b) Examine the parts of the instrument and find out their uses and names by the following description: The light from the window can be reflected up from a moveable **mirror** through a small hole or **diaphragm** in the flat plate or **stage,** upon which is to be placed the object to be examined. The observer looks through a **tube** to which the **lenses** are attached. The cylindrical **eye-pieces** slide into the top of the tube near the eye. The more complex **objectives** are to be taken from their brass boxes and screwed into the lower end of the tube near the object. At this lower end there is attached a swinging **nose-piece** to carry both objectives and make the changing from one to the other more convenient. The hole in the stage may be

made smaller by **diaphragms** which slip in, or by an **iris diaphragm** in the more elaborate instruments.

(c) As the microscopes used in any course may be from different makers, you should consult tables posted in the laboratory and giving the magnifications with the different combinations of lenses in the instrument you are using. Learn to recognize, by their numbers and the size of the lenses, the "high power" and "low power" eye-pieces and objectives. Examine a chart showing the parts of a microscope and how the light passes through the lenses.

(d) Clean thoroughly a glass **slide** and one of the thin **cover-glasses** by washing in weak alcohol and rubbing dry with a piece of cheese-cloth. With a pipette, put a small drop of water on the slide and with needles and forceps unravel a few hairs of woolen cloth in the drop. Handling the cleaned cover-glass with forceps, lower it carefully upon this drop. Place the slide thus prepared upon the stage of the microscope and direct the light through it. Screw the low power objective into the nose-piece and insert an eye-piece in the top of tube. To see the object clearly "**focus**" the lenses by moving the tube with its **coarse adjustment** until the fibres are clearly seen. More delicate focusing is obtained by the **fine adjustment,** a horizontal milled screw which is turned to the left (counter clockwise) to raise the tube; to the right (clockwise) to lower it. Compare the two sides of the mirror and in subsequent work see what results are obtained by the use of each side.

(e) After a few days' work with the instrument, a brief practical examination may be expected upon the following points: (1) Finding light with mirror, which is most easily done by removing the eye-piece and looking down into the tube through the low objective. (2) Finding the focal plane of an object and determination by focusing of the vertical spacial relations of its parts. (3) Adjustment of the diaphragm to different powers and objects. (4) Mechanism of the fine adjustment.

## II. RULES FOR USE OF THE MICROSCOPE

1. **Do not touch the glass of lenses; if they become dirty ask the assistant's aid in cleaning them with lens-paper.**
2. **Never unscrew the parts of the eye-pieces or objectives.**
3. **Use a low power first—a high power afterwards and only when necessary.**
4. **Do not use the high objective without a cover-glass over the object you have placed on the slide.**
5. **Use the high power eye-piece, only after the low one has been tried.**
6. **Do not leave lenses or mirrors exposed to direct sunlight.**
7. **With high powers, use a smaller hole in the diaphragm.**
8. **Learn to keep both eyes open when you are looking through the microscope.**
9. **Put the microscope into its case carefully, using care not to jam the mirror and nose-piece against the back of the box.**

# HISTOLOGY OF THE FROG

## I. THE BLOOD

(a) Bring a clean slide, a cover-slip and forceps to the centre-table where the instructor will give you a drop of the frog's blood. Place the slip upon this at once, lowering it with the forceps and examine with the low power of microscope. The numerous **corpuscles** will be seen floating in a clear fluid, the **plasma.**

(b) Examine with the high objective and determine the shape of the **red corpuscles.** As the blood stands look for the **white corpuscles** or **leucocytes** which are likely to attach themselves to the underside of the cover or the surface of the slide. Get a clear idea of the shape of these, then with a piece of oil-clay make a model of each kind of corpuscle. Demonstrate the model to one of the instructors.

(c) Stain with **aceto-carmine** or **methyl-green** in the following way: Using a slide having a fresh drop of blood covered with a slip, place a small drop of the stain on the slide near one side of the cover so it will be drawn under. This may be aided by touching the opposite edge of the cover with a bit of filter paper. After a moment or so draw off the stain with filter paper, using a drop of salt solution to help rinse away any excess. Look for the **nucleus** in each kind of corpuscle.

**Exercise 1.** Make a drawing of each kind of corpuscle. Size, about 2 inches in diameter.

(d) Examine a demonstration of the circulation in the web of a frog's foot, observing the flow of the corpuscles in the **capillaries.** Can you tell **arteries** from **veins?** Can you see the **pulse** in all the vessels? If the material is available, individual specimens of small fish or tadpoles, anaesthetized with chloretone, will be given out for individual study of the circulation in fins or gills. A drawing should be made, if such a preparation is examined.

## II. PAVEMENT EPITHELIUM

(a) Get from the centre-table a bit of the outer layer of the skin which frequently sloughs off from formalin specimens. Spread out flat in a drop of water on a slide, cover with a slip and look for the polygonal **cells** and their **nuclei.**

**Exercise 2.** Draw a portion of the skin showing a number of these cells. Size, about 1 inch across the cell.

(b) Understand that this pavement of flattened cells comprises only the outermost layer of the skin which is much thicker than this and made up of a variety of epithelial, gland, muscle and connective-tissue cells.

## III. CILIATED EPITHELIUM

(a) Get from the centre-table a bit of **mucous membrane** from the mouth of a recently killed frog. Place on a slide in a drop of salt solution and tease into small bits with your needles.

Put on a cover and examine with low and then with high power. Look for motion among the smaller particles and find out what is causing this. Find an individual **cell** which is separated out and on which you can see the **cilia.** Look also for the **nucleus.** If it cannot be seen in the fresh cells run in a little methyl-green or aceto-carmine.

**Exercise 3.** Draw a single cell or better a small group showing the above. Size, about 1 inch across.

(b) Observe in the demonstration at the centre-table how the cilia act upon small objects placed upon the surface of the mouth cavity.

## IV. COLUMNAR EPITHELIUM

(a) Get from the centre-table a bit of a frog's intestine which has been **macerated** by soaking in 35 per cent alcohol, or some other macerating fluid. Place on a slide in a drop of the fluid, and holding the piece with your forceps scrape off some of the **mucous membrane.** Discard the muscular portion of the wall and tease the bit of mucous membrane into very small particles. Put on a cover and examine with the low and then with the high power. Find the elongated **cells** each containing a **nucleus.** Look carefully for any structures in the nucleus. Some of them, **goblet-cells,** have a clear oval mass at one end or a space in the cell from which such a mass has been discharged. This oval mass is a drop of **mucus**, which was about to be secreted into the intestine.

(b) The nucleus can perhaps be made clearer by staining with **magenta.**

**Exercise 4.** Draw several of these cells, including if possible a good specimen of a goblet cell. Size, about 2 inches in length.

## V. STRIPED MUSCLE

(a) Cut from any of the body muscles of a freshly killed frog, a very small bit of the muscle substance. Put this **in a watch-glass half full of salt solution** and tease out with needles

until you can see with your eye the fibres of which it is composed. The fibres will be readily seen and **the teasing process must be stopped as soon as they come apart.** Care must also be taken not to crush the individual fibres. Put on a slide under a cover slip and examine with the low power. The long cylindrical fibres are bound together by connective tissue and each fibre shows a distinct **transverse striation.** There is a **longitudinal striation** which is less distinct. Examine a single fibre under the high power and make out the **sarcolemma** or membrane surrounding the fibre, and the numerous **nuclei.** Stain with methyl-green or aceto-carmine if the nuclei are not easily seen.

**Exercise 5.** Draw a portion of a single fibre showing these points. Size, about 1 inch in diameter.

## VI. UNSTRIPED MUSCLE

(a) This may be obtained from the urinary bladder or from the muscular layers of the digestive tract. Get from the centre-table a piece of this material which has been properly macerated. Tease out thoroughly and put on a cover slip. Find the spindle shaped **cells.** Stain with magenta if the **nucleus** is not readily made out.

**Exercise 6.** Draw a typical cell of this sort. Size, about 2 inches in length.

## VII. CARTILAGE

(a) Get with the aid of the assistant a thin bit of cartilage from the end of the sternum, or cut with a razor a thin section from the head of the femur of a freshly killed frog. Put on a slide in salt solution, cover with a slip and find the transparent homogeneous **matrix** containing numerous **cell-spaces,** or **lacunae,** in each of which is a nucleated cell. Observe here and there the groups of cells formed by **binary fission.** Stain with methyl-green or aceto-carmine.

**Exercise 7.** Draw a small area, showing several cells and the other points above noted. Size of cells, about one-half inch in diameter.

## VIII. CONNECTIVE TISSUE

(a) Carefully separate two of the muscles of the leg of a fresh frog and note the delicate web of connective tissue between them. Or, note the fine strands of connective tissue between the skin and the muscles of the body wall. With fine forceps lift up a small shred of this, snip it off with scissors, and place it on a **dry slide.** Then, with two needles, spread it out into a thin layer, breathing on it to prevent drying. Place a cover-glass upon the preparation and then let a very small drop of salt solution creep under the cover and moisten the tissue. The reason for this procedure is that if connective tissue is placed in fluid it contracts into a lump, too opaque for examination, and cannot be again spread out.

(b) Examine first with the high and then with the low power and note two kinds of fibres, broad crinkly ones the **white fibres,** and narrow branched ones the **elastic fibres.** Scattered among these are nuclei. Stain with methyl green.

**Exercise 8.** Draw, showing the above.

## IX. BONE

(a) Pieces of dried bone, ground to thin sections, will be used. In these only the inorganic substance of the bone remains, but the extent and distribution of the **bone cells** is shown by the cavities which the cells once occupied. These cavities all appear black, because in the grinding of the section they become filled with air and dirt. Examination with low and high power will show elongated black areas, the **lacunae,** or spaces once occupied by cells, and radiating out from these fine black lines the **canaliculi,** which in life are occupied, in part at least, by delicate processes of the bone cells. Compare the structure here observed with that seen in cartilage.

(b) In some bones, the cells are grouped about canals in which run blood vessels. These are termed the **Haversian canals,** each of which with its surrounding cells is an **Haversian system.**

**Exercise 9.** Draw several lacunae and their canaliculi. Size, about 1 inch for the length of a lacuna.

## X. WALL OF DIGESTIVE TRACT

(a) The cells comprising the wall of the digestive tract may be studied **in place** by preserving a small section of the tract and cutting this into very thin sections. As the process by which such objects are made ready for cutting is somewhat elaborate, material will be provided already embedded in paraffin and will be cut for each student by one of the instructors. Or, permanent slides may be used, in which case great care should be exercised not to break covers or even to press upon them with fingers or objectives.

(b) If the material is to be cut for each student, clean a slide **very thoroughly,** as this is to be a permanent mount, smear on one side toward the middle of the slide, and over an area about the size of your cover-slip, a small amount of albumen fixative. Allow a drop of distilled water to spread evenly over the smeared area. Place the "ribbon" of sections on this water and draw off the excess of water with filter paper. See that the ribbon is in such a position as to give a neat appearance and paste a gummed label on one end of the slide. The preparation should now be set aside until the water has all evaporated, 24 to 48 hours being sufficient if the slide is kept in a warm place.

(c) When all the water has evaporated, place the slide in a jar of **xylol** for several minutes, to dissolve the paraffin, during which time a cover-glass may be carefully cleaned. As quickly as possible after this, place the slide **right side up** on a clean piece of filter paper. Add a drop of balsam before there is time for the xylol to evaporate and then lower the cover glass into place with forceps, being careful not to include air bubbles. If the cover glass does not have its sides parallel with those of the slide, move it into this position so that when the slide is permanently dry it will present a neat appearance. Wipe off any xylol remaining on either side of the slide and the preparation may be studied at once, if care is exercised not to press down on the cover with the objectives or otherwise, before the balsam has set hard. **Even when hard, a similar precau-**

**tion should always be exercised in handling this or other slides, as such preparations represent many hours of work and are easily ruined through careless handling. Another precaution is the keeping of the slides in a horizontal position as long as the balsam is thin enough to flow. This may be for a period of several weeks. You should therefore always keep such preparations in the regular slide boxes provided for the purpose and the box on end.**

(d) Examine with low power identifying the layers of **peritoneum, longitudinal muscle, circular muscle, sub-mucosa,** and **mucosa,** comparing them with the figure made from a transverse section cut with a scalpel. Studying further with low and high objectives, note the peritoneum, which is a pavement epithelium, here seen edgewise; the layer of longitudinal muscles, here with its cells cut transversely; and the circular muscles, here cut lengthwise. Recall text-book or lecture notes upon the cells of smooth muscle. The sub-mucosa is composed of connective tissue, blood vessels, etc. The mucosa shows typical columnar epithelial cells, some of which may be **goblet-cells** with their drops of mucous. If the stomach is being studied the **gastric glands** will be seen.

**Exercise 10.** Make a figure, about 2 by 5 inches, which will show a small part of the circumference and all the layers. This figure should show not merely the several layers, but also the details of their cells as they appear in the section studied and should accurately represent what appears in your particular preparation.

## XI. SECTIONS OF SKIN

(a) Sections cut at right angles to the surface of the skin may be prepared in the same way as the sections of the digestive tract. Examine such a section with low power making out the two layers, **epidermis** and **dermis.** The dermal portion has conspicuous **glands.** With the high objective, find the layers of cells composing the epidermis. Where is the **pavement epithelium** of your previous study? Find how the cavities

of the glands open to the outside. Are the cells lining the glands derivatives of the dermis or the epidermis? What is the direction of the connective tissue fibers in the dermis? Do you see anything like **blood vessels?** Note the position of any **connective tissue nuclei** and **pigment cells** in the dermis.

**Exercise 11.** Draw a section of the skin showing the above points. The figure should be made at least 3 by 4 inches to enable you to show the cells properly and should be drawn accurately for the details of your particular section.

## UNICELLULAR FORMS

### PHYLUM, PROTOZOA

### I. THE "PROTEUS ANIMALCULE," (AMOEBA PROTEUS)

Amoeba is frequently found in fresh water and is often obtained for laboratory study by placing pond plants in a dish and allowing them to stand. After such a culture has remained undisturbed for several days, the scum which forms on the surface of the water or against the sides of the dish should be examined for amoeba. For this laboratory work, slides of such material freshly mounted and known to contain amoeba will be given out.

(a) Use a very small aperture in the diaphragm and look with the low power objective for transparent objects having much the appearance of extended white blood corpuscles, but considerably larger. After finding such an object examine under high power and if it is an amoeba you will recognize the flowing movements seen in the white corpuscles.

(b) Watch the changes in shape. Does the animal accomplish a definite locomotion by this means? The processes which flow out from the amoeba are called **pseudopodia,** i. e., **"false feet."**

**Exercise 1.** Make four drawings showing the outlines assumed by a single amoeba at intervals of one minute.

**Exercise 2.** Begin a large drawing of an amoeba by making an outline 3 to 4 inches across and add to this the items noted in the following sections as you come to them.

(c) Examine the structure of the substance composing the amoeba. There is an outer region of clear **ectosarc** and an inner mass of granular **endosarc.** Recall the condition in the white corpuscles of frog. Some of the larger masses in the endosarc will be found surrounded by a vacuole. Sometimes the contents of such vacuoles can be recognized as green plant cells similar to the ones found living in the water outside; or if specimens can be successfully fed on carmine the red particles will be found in many of the vacuoles. These spaces are **vacuoles of digestion** or temporary stomachs in which food particles are being digested. Examine the various granules of the endosarc with a view to determining their size, shape and nature. What is the nature of the boundary between ectosarc and endosarc?

(d) A single larger vacuole may be identified as the **contractile vacuole** if it is seen to contract and reappear.

(e) The **nucleus** is best made out by looking carefully among the vacuoles of a large specimen for an oval mass with finely granular transparent contents and about the size of the contractile vacuole when expanded. Examine demonstrations of stained amoebas. Does the nucleus of the living amoeba change its shape?

(f) If specimens are carefully watched, it is sometimes possible to observe the manner in which the food is ingested and the fecal matter egested.

**Exercise 3.** Complete the large drawing by putting in all these details and labeling thoroughly.

**Exercise 4.** Study the contractile vacuole as it appears and disappears and represent this by diagrams.

**Exercise 5.** Study the movements of the cytoplasm by which single pseudopodia are formed and withdrawn and represent by drawings.

**Exercise 6.** Examine amoebas in a drop of water on a slide, but without the cover glass and under the highest magnification obtainable with the low objective. What can you make of the third dimension? Correct any errors in your previous figures and make a clay model to show the superficial features of the amoeba.

(g) Understand from lectures and text-book what is known regarding the life history of the amoeba.

## II. EUGLENA VIRIDIS

This is another form which frequently occurs in fresh water, being some times present in such numbers as to cause the green or reddish color in the ooze at the bottom or in the scum floating on the surface of ponds and sluggish streams.

(a) Clean a slide and cover and get a drop of the material containing the euglena. With the low power, look for elongated green bodies which may be at rest or slowly moving about. Put one under the high power and observe the form and movements. Can you distinguish an anterior and a posterior end? Look carefully for the structure which causes the locomotion. Find specimens which are expanded and others which are contracted; or better, observe how a single specimen may expand and contract.

**Exercise 1.** Make a clay model to show the external features.

**Exercise 2.** Draw on a large scale, 3 to 4 inches long, the outlines of a contracted and of an expanded specimen and as you make them out add the following details to the latter drawing.

(b) Continue, examining a favorable specimen. Do you find anything like a **nucleus?** Anything like a **contractile vacuole?** What other structures can you find inside or on the outer surface? At the anterior end a spot of red **pigment** will be observed. Look at this end for a small notch in the outline of the body. This marks the position of the **gullet.**

(c) Crush the specimen by pressing on the cover with handle of scalpel or needle while you watch with the low power, or you

can place your slide on the table, lay a piece of filter paper over the cover and press down with finger. Examine a specimen thus crushed and see what you can find out regarding the substance of which the euglena is composed, i. e., whether fluid or solid, granular or homogeneous, etc.

(d) Prepare another slide and stain with strong iodine or methyl-violet. Look for the **flagellum** at the anterior end and add this to the diagram if not already made out.

**Exercise 3.** See that your drawing of the expanded specimen contains all the points made out and is thoroughly labeled.

**Exercise 4.** Study and draw encysted euglenas if such material is available.

## III. THE "SLIPPER ANIMALCULE," (PARAMOECIUM CAUDATUM)

When hay or some vegetable debris is soaked in pond water for a time a scum collects on the surface and after some days numerous moving white specks may appear around the edge and at the surface of the dish, often these will be found to be paramoecia; or the animals may be found in abundance in small stagnant ponds where the water has a foul odor.

(a) Place a small drop from such a culture on a clean slide. Examine first with the low power and without a cover and note the rapid movements, characteristic shape and colorless body.

(b) Put a very small amount of absorbent cotton fibres upon the drop of water and then a cover-slip. The animals will thus be caught in little pens formed by the meshes of the cotton and may be kept within a limited space. Find a specimen which is thus enclosed, but not in any way crushed and study with low and high powers. Study the locomotor activities. Does the paramoecium act as though it might have volition? What determines its movements?

(c) What is the shape? How does the anterior end differ from the posterior? As the animal becomes quieter make out the **cilia** which cover the surface of the body and cause the locomotion, on one side the **buccal groove** leading into the

substance of the body, the **contractile-vacuoles,** two clear vesicles which appear and disappear, **food-vacuoles** scattered through the body and having variously appearing contents.

**Exercise 1.** Make a model of the animal to show its external features.

**Exercise 2.** Make a drawing, 3 to 4 inches long, showing only the outer surface of the body and the cilia.

**Exercise 3.** Begin a large sketch, 3 to 4 inches long, showing the animal in optical section. Put in all you have thus far observed and add other points as you find them.

(d) Make a fresh mount without cotton and stain with **methyl-green** or **aceto-carmine.** Look for a **nucleus.** Does the nucleus react differently from the **cytoplasm** when the stain is applied? What do you think the staining indicates regarding the chemical or physical composition of nucleus and cytoplasm? A smaller **micro-nucleus** can be demonstrated by more accurate staining methods. It is embedded in a depression at one side of the larger **micro-nucleus,** which is the one you see, and is not likely to be seen by the staining methods here used.

(e) Look in this preparation for specimens in which many stiff processes much longer than the cilia have been extruded from the body as a result of contact with the stain. If you do not find them try another slide, adding a drop of stain to the water on the slide before putting on the cover. These are the **trichocysts** which are used for defensive purposes.

**Exercise 4.** Draw, on a large scale, an optical section of a small portion of the body margin, showing trichocysts and cilia.

(f) Take a very small drop of water containing some of the animalcules and add an equal amount of water containing finely powdered carmine, or India ink. Watch carefully for some time and see whether any carmine or ink gets into the body, where and how. Study also the action of the locomotor cilia as they drive the particles of carmine about. Examine any carmine which has entered the body and understand how the food vacuoles originate.

(g) Mount some specimens in a very small drop of water and hold in place by the weight of the cover-glass. Study the

formation and collapse of the **contractile vacuole** and time the contractions.

**Exercise 5.** Write an accurate description, accompanied by three drawings, showing stages in the process.

(h) Put a small drop of water containing paramoecia upon a slide and cover with a slip. Draw off the water with filter paper until the animals are brought under pressure, but not crushed. Examine a specimen with the high power and look along the margin of the body for rod-like bodies, the **trichocysts** before discharge. The firm line outside these is the **cuticle.** The trichocysts lie in the region known as the **cortex.** Draw off the water until the animal is crushed and the semi-fluid **medullary region** of the protoplasm flows out. Examine this mass with your highest power and see what you can make of it.

**Exercise 6.** Complete the drawing of an optical section, adding as many details as possible.

**Exercise 7.** Study and draw stages in **binary fission** and in **conjugation** if these can be found in any of the cultures.

## IV. PARASITIC PROTOZOA (GREGARINA AND MONOCYSTIS)

Many protozoa live as parasites in the bodies of other animals. Notable among these, are the **gregarines,** which occur in the digestive tracts of many arthropods. Material for this study is readily obtained from the larvae or adults of the meal beetle, Tenebrio.

(a) Take one of the living larvae of the beetle upon a slide and cut off the tips of the body, not quite severing the head, so that it may be pulled away and the entire digestive tract of the animal drawn out. Examine with microscope the contents of the tract through its transparent wall without a cover-slip and look for individuals which are heavily infected. When found, the tract may be chopped or teased to pieces and the material distributed on several slides. Or, if the parasites are not abundant, each tract may be at once teased and covered with a slip. Avoid getting much of the fat-body of the insect

mixed with the bits of the digestive tract and do not use salt solution unless necessary as the gregarines live longer when in the fluid of the gut cavity.

(b) Look for organisms with sharp outlines and definitely divided into two parts, **protomerite** and **deutomerite.** Where is the **nucleus?** Is there a **cell wall?** What is the nature of the **protoplasmic structure?** Do the organisms move and how? Do you find more than one type? Are any specimens attached in any way?

**Exercise 1.** Draw figures of good size representing the above.

(c) Understand the complete life-cycle of such a form, particularly the stages of **spore formation** and **conjugation.** See demonstrations of these stages in **Monocystis** a parasite from the seminal vesicles of the earthworm, and draw if time allows.

## V. YEAST AND BACTERIA

(a) Clean three test-tubes. Fill the first test-tube two-thirds full with distilled water; the second and third two -thirds full with **Pasteur's solution.** To each tube add a little yeast culture. Boil the second tube after plugging with cotton. Set all three tubes aside in a jar.

**Exercise 1.** Observe and record any changes visible to the unaided eye in the course of several days.

(b) Take some of the solution from each test-tube, place on a slide covered by a slip and study with microscope. Do you find the **"yeast plants"** in each test-tube? Why?

(c) Get material from the yeast culture at the centre-table. Study and make out the general shape of cells, granular content, **vacuoles, cell wall,** methods of **growth** and **reproduction.** Can you find the **nucleus?**

**Exercise 2.** Draw a single cell, size about 1½ inch in diameter showing detailed structure and a colony or group of cells in outline only.

(d) Take potatoes that have been in steam sterilizer for one hour. Cut in halves with a knife, that has been heated in a

flame. Lay halves directly upon the surface of a sterilized glass plate, with cut surface upward. Be sure that nothing except heated knife touches cut surface. Leave potatoes exposed to air of laboratory for one hour, at end of this time cover the potato with a sterilized finger bowl. Paste label on glass plate and finger bowl with your name and Lab. Section. The potato will remain on your table for several days. Each time you come into laboratory examine the surface for any changes that are visible to the eye. When growths appear scrape off a little of the material, dilute with sterile water and examine under the microscope.

**Exercise 3.** Record the changes from day to day as seen with the eye and under microscope.

(e) Get a small drop of water containing carmine or india ink particles and examine under high power; note the motion of the smallest specks. This is called "Brownian movement." Is it a translation or a vibration?

(f) Examine a drop of hay or other turbid "infusion" and find the cause of the turbidity. Remove the most of the water from under the cover and study the objects with a good light and high power. Is there any Brownian movement? Is there any active translation from place to place? Focus carefully and see that each minute body appears light or dark according to focus.

(g) Study a fresh slide and make out what you can as to the form and proportions of the different objects. Can you make out any nucleus or other internal structure. These small objects are **Bacteria.** What grounds can you give for supposing them to be cells?

**Exercise 4.** Draw outlines, on a very large scale, showing the shapes of as many distinct types as you can make out.

(h) Examine the various cultures and infusions which are on the centre-table and get an idea of the kind of material in which bacteria in the active state may be very abundant. Do you think they are more widely distributed in their active or their resting condition?

(i) If time allows try to find good cases of "spore formation" and of "zooglea." Draw.

# THE HYDRA. (HYDRA VIRIDIS, OR H. FUSCA.)

PHYLUM, COELENTERATA. CLASS, HYDROZOA.

## I. HABITAT AND ACTIVITIES

The hydra occurs in quiet pools containing leaves and other decaying vegetable matter. When such material is brought into the laboratory and allowed to stand in a glass jar many of the animals will crawl out and attach themselves to the leaves and water plants. There occur commonly about Columbia two species; **Hydra viridis,** which is green in color and quite small, and **H. fusca** which is larger and of a brownish tinge.

(a) Examine jars containing specimens of hydra and note whether they tend to collect on the lighter side of the vessel. In size they will be found to vary from very short ones up to those about one-fourth inch in length. Examine several specimens in the jar and see how the free end differs from the attached. Are they found in colonies or as individuals? Do any have side branches? If such cases of "budding" are not found among the living specimens ask for a slide showing this condition. The buds are only temporarily attached to the parent, each becoming eventually set free and beginning an independent life.

(b) With the aid of the assistant, transfer several with a pipette to a clean watch glass containing water from the jar. How firmly are they attached? Examine with the eye and with a hand lens against the dark background of the table. Touch with a needle and note the result. Do any of the hydras attach themselves to the glass?

(c) The process of feeding may be studied next, or it may be deferred until the completion of section II below. Take an extremely small bit of fresh meat, which you can get by teasing out on a slide in a drop of water some lean beef or a piece of some fresh-water animal, and with a needle push it into contact with the free end of a hydra. If it adheres to

the tentacles, examine the specimen under the low power of the microscope. If you are unsuccessful with the first after several attempts try other specimens, until you find one that will take food. Watch the process carefully.

**Exercise 1.** Make several drawings of good size, 2 to 3 inches long, to show the feeding.

## II. GENERAL EXTERNAL CHARACTERS

(a) Examine with low power of the compound microscope and notice the cylindrical body, which varies much in length and is attached at one end. At the opposite end is a conical projection, the **hypostome,** around which are several **tentacles.** How many? The tentacles are covered with knob-like swellings at intervals varying with the state of contraction. Short developing tentacles will sometimes be found.

(b) The **mouth,** at the tip end of the hypostome, is round when widely open. What shape has it when closed? The **base** of attachment is the thick end by which the animal adheres to a support. Sections show that the cells of the base are glandular and secrete a sticky substance, by which the hydra attaches itself.

(c) Compare the structure of a bud with that of the parent animal.

(d) Examine now the body of the animal; it is made up of a transparent outer portion, the **ectoderm,** and an inner colored portion, the **entoderm.**

**Exercise 2.** Make a detailed drawing, 2 to 3 inches long, of an expanded specimen to show the above points and an outline of a contracted one.

## III. INTERNAL STRUCTURE

(a) Using a pipette, transfer a specimen to a slide. Put on a cover, **supported at one side only, by a very small piece of clean filter paper** which should first be dipped into water. Examine with the low and, for the finer points, with the high

power. Be careful not to crush. Make out the following points, which can be done most successfully if the specimen is well expanded. The transparent ectoderm will be found to surround the colored entoderm and the latter to surround a central cavity. Look for particles in this cavity. This is the **enteron** or **digestive cavity.** Is there anything to indicate that the tentacles are also hollow? If so, are their cavities in free communication with the main enteron?

(b) Certain clear oval bodies, the **nematocysts,** are present in the ectoderm. In what regions of the body are they found and where are they most abundant?

(c) Projecting into the water near the nematocysts are fine hair-like processes, so delicate and transparent they are easily overlooked. They are called the **cnidocils.**

(d) Remove the bit of filter paper and press gently on the cover with a needle until the hydra is crushed, and look for large nematocysts with threads projecting from them. Compare these with some that have not been discharged. Look for nematocysts embedded in their cnidoblast cells, and for the different types of nematocysts.

**Exercise 3.** Draw on a large scale the large barbed type of nematocyst, as it appears before and after discharge. Size, about 1 inch for diameter of bulb.

(e) Mount a fresh specimen under a cover and supported with filter paper as before and observe the tentacles under a low power as aqueous **safranin** is run under the cover glass. See if filaments are discharged and how they look when compared with the ones seen above. Remove the filter paper and with the high power make out the origin of these filaments. Understand from your lectures and the text-book the use of these organs. Recall the trichocysts of paramoecium.

**Exercise 4.** Draw one of the smaller types of nematocyst before and after discharge. Size, proportionate to Exercise 3.

(f) Prepare a fresh slide, supporting the cover with filter paper as above, and see if you can discover cell outlines in the ectoderm or endoderm of any part of the body. Review with this specimen any other points you have in doubt.

(g) Study prepared cross-sections of hydra. Note the large central digestive cavity or **enteron,** also the body wall composed of ectoderm and endoderm and between them a distinct line marking the **supporting lamella.**

**Exercise 5.** Make a diagram of the entire section in outline only to show these facts. Size, 4 to 5 inches.

(h) Study under the high power and make out **nuclei, nematocysts, vacuoles,** the lines of division between the cells and any other details which may be found.

**Exercise 6.** Add these details of ectoderm and endoderm to a small part of the section shown in Exercise 5.

(i) The shape of the individual cells will be much better understood after studying the macerated specimens. To do this, place a hydra on a slide with a very little water, and add a drop of Bella-Haller's fluid. After one or two minutes draw off the fluid with filter paper and add a drop of strong aqueous methyl-violet, which should stand about the same length of time. Draw off the stain with filter paper, add a drop of water, break up the specimen by a little teasing and put on cover glass. Find the several types of cells. If necessary separate the masses of cells still further by tapping very gently on the cover glass with a needle. The **large endoderm,** the **large ectoderm,** the **interstitial,** the **gland cells of the endoderm** and the **cnidoblast cells** can be made out in the best preparations. Understand the position of the interstitial cells and their relation to the cnidoblast cells.

**Exercise 7.** Draw a typical cell of each kind made out.

## IV. SEXUAL REPRODUCTION

If specimens are available, study live individuals having male and female organs and make drawings, also sections showing eggs or young embryos in the ovaries and the testes containing spermatozoa.

## V. GENERAL POINTS

Keep hydras for some days in a tumbler, or bottle, near a window and with water fleas or other forms upon which they may feed. Watch the process of catching and eating the living prey. Watch hydras that are budding and see if the buds separate and form new individuals. Get some way of marking on the outside of the dish the place of the hydra's attachment and see whether it changes from day to day. Cut several hydras into pieces and see if there is "regeneration." In making this last experiment use a watch-glass which has been thoroughly cleaned and cover with another, or a larger glass dish.

# HYDROID COLONIES

## (OBELIA GENICULATA AND PENNARIA TIARELLA)

Hydroids are marine animals closely resembling hydra in their plan of structure. Budding occurs extensively and the buds instead of dropping off remain connected with the parent stem, forming a colony. In many hydroid colonies there is a **"physiological division of labor"** between the units of the colony so that some become nutritive and others reproductive individuals. For the study outlined below specimens preserved in formalin will be used.

(a) Examine museum specimens of **Obelia geniculata** to determine the extent, attachment and mode of vertical and horizontal growth of the colony. Examine several of the vertical portions in a watch-glass of water with lens or low power and note general resemblance to a budded hydra.

**Exercise 1.** Draw, on a scale of 2 or 3, a single one of these vertical parts, showing how it arises from a horizontal stem. So small a figure cannot, of course, show the details and is to show only the main parts of the colony and manner of branching.

(b) Mount one or more of these vertical parts on a slide in a drop of water, selecting with aid of an instructor one which has formed its reproductive units. See that the specimen is properly expanded before putting on the cover, and support the cover at one side with a bit of filter paper. Notice under low power the mode of branching and the individuals of the colony, **polyps,** at the ends of the branches. Do you find developing polyps or **buds?** Compare a single polyp with a single hydra, noting **mouth, hypostome, tentacles, ectoderm, endoderm** and **enteron.** Each polyp is surrounded by a cup formed from an extension of the transparent **perisarc** which covers the stem of the colony. Are there any modifications of the perisarc which would make it more flexible? The core of living material within the perisarc is called the **coenosarc.** Can you find any indication of ectoderm, endoderm and enteron in the coenosarc of the stem? With what part of a hydra is the stem comparable? How does food pass to a growing bud, or to the horizontal extensions of the colony? The type of individual most numerous in the colony and found toward the free ends is the vegetative or feeding unit and known as a **hydranth.**

**Exercise 2.** Draw, on a large scale, a single hydranth showing its connection with the main stem.

(c) Near the bases of the vertical stems are the reproductive polyps, the **blastostyles** and **medusae.** The former have large cups of perisarc with a small opening at the free end, and their core is a continuation of the coenosarc. They are comparable to polyps without mouths and tentacles. They produce, by budding, the peculiar polyps known as the **medusae.** These medusae become detached, much as do the buds of a hydra, as soon as they are fully formed and, passing through the small opening at the end of the cup, swim away in the water. Examine a demonstration of these medusae of obelia. There are therefore three kinds of units in this colony, all formed by budding: (1) Feeding polyps or hydranths, (2) blastostyles and (3) medusae.

**Exercise 3.** Draw a single blastostyle showing its connection with the main stem, as in Exercise 2, and medusae in different stages.

(d) Examine museum specimens of other hydroid colonies, noting general shape and appearance, as determined by size of the hydranths and nature of the perisarc. For detailed comparative study, the hydroid **Pennaria tiarella** may be used. Examine a branch of the colony in a watch glass with lens and low power of microscope. How and where does **budding** occur? How do the hydranths differ from those of obelia? What is the relation of the ectoderm and endoderm in the tentacles? Where are the medusae buds? How many kinds of units are present in the pennaria colony when compared with the obelia as explained in paragraph (c)? Compare these two hydroids point by point and determine the relative amount of specialization which the parts exhibit. On the whole, which species would you consider the more highly organized?

## A HYDROID MEDUSA (GONIONEMUS MURBACHII)

Since the medusae of **obelia** are very small we shall examine a larger form, **Gonionemus murbachii,** which originates from a hydroid colony* in a similar manner.

(a) Examine a specimen in a watch-glass under water. Notice the numerous **tentacles,** the shape like a flat bell with a clapper, the **hypostome,** and a shelf, the **velum**, projecting in from the margin. The **mouth** is at the end of the hypostome. We speak of **oral** and **aboral** sides, not of ventral and dorsal. Protruding upon the concave oral surface are four convoluted ridges, the **reproductive organs,** from which the eggs and sperm are shed directly into the water. On the oral surface above each reproductive organ is a **radial canal,** extending from the central **stomach** to a **circumferential canal** at the bases of the tentacles. These canals are a modification of the simple enteron of an early stage in the medusa's development. Each tentacle has a core of **endoderm** arising from the circum-

*The life-cycle of gonionemus is not completely known, but a period of budding comparable to the hydroid phase of obelia seems to occur.

ferential canal and is covered with **ectoderm,** as is the entire outer surface of the animal. At the base of each tentacle is a colored **eye spot.** Examine the specimen in the watch-glass from the aboral side under low power, looking along the circumferential canal for clear vesicles, the **lithocysts** or organs of equilibration, and for small **developing tentacles.**

**Exercise 1.** Draw the medusa from an oral view. Size about 3 to 4 inches across bell.

**Exercise 2.** Draw a vertical section in the plane of the radial canals. Size the same as in Exercise 1.

(b) Much of the above can be understood completely only in connection with the explanations in lectures and text-book. Understand from these the structure of the medusa as compared with the hydranth, and the "alternation of generations" by which the medusa produces the hydroid colony and the colony the medusa.

## THE EARTHWORM (LUMBRICUS HERCULES)

PHYLUM, ANNULATA. CLASS, OLIGOCHAETA

### I. THE LIVING ANIMAL

(a) Place a vigorous, active worm upon wet filter paper in a dissecting pan and carefully observe the mode of locomotion. How does it elongate and contract? Can you see stiff spines projecting from the sides? Can they be drawn in? Is there a rhythm in these changes? Draw the worm through the fingers and feel the spines, or **setae.** How many are there on one ring? Place the worm on its back. Does it right itself? Will it crawl backwards? Compare the anterior and posterior ends, the dorsal and ventral, the right and left sides. Which are alike? Touch various parts of the worm to see which seem the most sensitive. Note the movements of the soft lobe above the mouth, the **prostomium.** On the mid-dorsal line

look for the blood vessel which shows through the skin. Does it pulsate? Which way does the blood move? Hold up the worm to the light and see the dark central axis which is the digestive tract with its contents. On the ventral side may be seen light colored swollen areas, the **skin glands.** Those on some segments may be associated with a smooth swollen band passing around the animal, the **clitellum.** On the 15th segment, swellings mark out transverse slits on each side, the openings of the **vasa deferentia** or ducts for the discharge of sperm. On the 14th segment in a similar position are the two very small openings of the oviducts. Count the entire number of segments or **somites** and record result, comparing with counts in neighboring specimens.

**Exercise 1.** Draw the anterior end, as far back as a point just behind the clitellum, on a scale of 2 or 3 and from a ventral or a side view.

**Exercise 2.** Draw the posterior end on same scale, showing about 10 or 12 segments and from a ventral view.

## II. GENERAL INTERNAL STRUCTURE

(a) For this, study a freshly killed or a preserved worm. Fasten down in a dissecting pan, dorsal side up, by pinning through the first somite and again toward the posterior end. Make an incision about one and one-half inches long, just back of the clitellum and **on the mid-dorsal line,** but do not cut too deep. Using fine scissors cut toward the head end with great care not to cut deeper than the wall of body and to keep on the dorsal mid-line. Spread out the edges of the cut body wall and pin them apart after breaking the transverse partitions, **septa,** which connect the inner face of the wall and the outer surface of the gut. **Slant the pins outward to give room for fingers and instruments in working.**

(b) You will now be able to see the brownish **intestine** and on its upper surface the large **dorsal blood vessel.** Toward the head, the gut becomes differentiated and is partly hidden by other organs which will be indicated presently. The skin

is made of two layers, the outer, colored part is the real skin, the white inner part is the muscle; both together form the **body wall.** Between the body wall and the digestive tract is a space, the body cavity or **coelom.** Thin lines pass from the digestive tract to the body wall across the body cavity. With a hand lens and a needle one may see and feel that these are the partitions, or **septa,** dividing the body cavity into chambers one behind the other. What is the relative position of septa and external rings?

(c) Continue the cut forward through the second anterior ring. Carefully separate the edges of the cut and see the different regions of the digestive tract. Identify the following: **pharynx, oesophagus, crop, gizzard,** and **stomach-intestine.** In the sexually ripe animal there are large yellowish-white lobes on certain rings; these are the three pairs of **seminal vesicles.** They more or less hide the oesophageal region of the digestive tract. There are five pairs of large lateral blood vessels, the **hearts,** which in the living animal may be seen to pulsate. On top of the digestive tract in segment 3 is a small white body, the **brain.** Spread out the body wall right and left and pin it to the wax, slanting the pins obliquely outward as before. In doing this break or cut some of the septa with a needle or scissors. How do the anterior septa differ from the others? Have they any different use? In all but a few of the anterior chambers of the body cavity there are paired fluffy masses on each side. These are the **nephridia** or excretory organs. With a lens look for fine blood vessels on these organs. Turning a part of the intestine to one side you may see these fine vessels connected with a median ventral vessel. Beneath the median ventral vessel is a large band, the nerve-cord. Cut the specimen open for its entire length and carefully separate the various organs to see them more clearly. Which segments are differentiated? Which ones merely repetitions of similar organs?

**Exercise 3.** Make a full page diagram of the region from prostomium to beginning of stomach-intestine to show all the organs thus far made out. The length of the segments may

be exaggerated and the organs drawn as if separated by dissection.

(d) Lift up the oesophagus with forceps, carefully cutting its attachment to the septa. Cut it across near the pharynx and pull it gently back, while cutting off the septa. How are the seminal vesicles placed with reference to the gut? Be careful not to remove the seminal vesicles from the worm while continuing to pull back the digestive tract and to cut the septa. Continue this as far back as the beginning of the stomach-intestine and so lift up and remove from the worm the oesophagus, crop, gizzard and part of the intestine in one piece. Examine this removed portion of the tract under water and correct any errors in your previous drawing. Find the **calciferous glands,** three pairs of lateral pouches on the oesophagus in the region hidden by the seminal vesicles. Cut open lengthwise, wash out and note the character of the lining and contents in each region of the tract. If a preserved specimen has been used, set aside the undissected portion for the subsequent work.

## III. COELOME AND NEPHRIDIA

(a) Clean a slide and cover and, with the aid of an instructor, draw out a drop of the **coelomic fluid** by means of a capillary pipette. Place immediately upon the slide, adding salt solution if necessary, and examine under high power. Find the **white corpuscles.** What are their characteristic activities? What organism do they resemble? Have they nuclei?

**Exrcise 4.** Draw, showing characteristic shapes. Size, 1 to 2e inches across cell.

(b) Using the preserved specimen dissected under Section II, cut out with fine scissors part of a septum with nephridium attached and examine under low and high power. The nephridium is a convoluted tube with interlacing blood vessels. In the larger muscular part there may be parasitic worms. Look for the ciliated funnel, or **nephrostome.** Understand the function and manner of action of the nephridium. With

the aid of an instructor, obtain a bit of a living nephridium, or better, one that is complete. Look for the peculiar flickering movement of the **cilia** within the tubule and study this with the high power.

**Exercise 5.** Draw the nephridium in whole or in part as observed.

## IV. THE REPRODUCTIVE ORGANS

(a) Using the specimen dissected in Section II, wash off the region of the reproductive organs by gentle currents from a pipette and examine the posterior face of the septum between segments 12 and 13. Under a hand lens the two **ovaries** may be seen lying one on either side and near the nerve-cord. Immediately behind each ovary is an **oviduct,** seen as a whitish area on the front face of the septum between segments 13 and 14, and in segment 14 as a fine cord which is very short and passes diagonally outward to its place of exit on the ventral body wall. Locate these parts without much pulling away of the remains of septa and nephridia and then make them more clear by gently pulling or cutting any tissue which renders them obscure. Examine a model and understand how the eggs pass from ovary to oviducts.

(b) Examination of the seminal vesicles, which should still be uninjured, will show that the three lobes which extend up on either side of the oesophagus are united by a common median region which lies below the gut and against the ventral body wall. A little picking away of this middle region will disclose four large bodies, rather indistinct in outline, but different in texture from the vesicles and resembling crumpled bits of paper. These are really greatly modified **funnels** which lie at the beginning of the male ducts, or **vasa deferentia.** Looking on the ventral body wall and outside the seminal vesicles, it is possible to find a fine duct running out laterally from the region of each funnel. The two on a side unite and pass straight back to the opening of the vas deferens on segment 15. By means of these funnels and ducts

the sperms pass out of the seminal vesicles. The sperm funnels really open within the closed cavity of the seminal vesicles. The sperms originate from bodies somewhat similar to the ovaries and in the same relative position in segments 10 and 11. Although these bodies, which are the true **testes,** are immediately in front of the sperm funnels, the sperms upon dropping from the testes do not at once enter the funnels, but pass up into the lobes of the seminal vesicles where they develop to mature spermatozoa, which are then ready to enter the funnels and vasa deferentia and so pass to the outside. The four testes and the four funnels have therefore a relation to the coelome similar to the ovaries and their oviducts, while the seminal vesicles by enclosing both testes and funnels in a common cavity prevent the spermatozoa from entering the coelome and furnish a place for their later development.

(c) The **seminal receptacles,** which should not be confused with the seminal vesicles, are small whitish bodies attached to the ventral body wall on either side in the region of segments 9, 10 and 11. They open to the outside only and their function is to retain the spermatozoa derived during copulation from another worm and which fertilize the eggs of this individual.

**Exercise 6.** Consult a model, or reference figures of the entire system, and then construct a semi-diagrammatic figure which will show all these parts. Review their relationships by tracing the course of the ova and spermatozoa from their origin to the external openings of oviducts and vasa deferentia.

(d) Carefully cut out one of the ovaries and transfer to a slide. Add a drop of glycerine, put on a cover and study under the low power. The **ova** will be seen in various stages of development. Where are they most advanced? The largest ones show clearly a **nucleus** and **nucleolus.**

**Exercise 7.** Make a drawing 2 or 3 inches in length showing the entire ovary.

(e) Understand from lectures and text-book the functioning of the various parts in copulation and egg laying. Exam-

ine again in a whole worm the **clitellum,** the markings which extend forward from this to the openings of the vasa deferentia and the external openings of the oviducts.

## V. NERVOUS SYSTEM

(a) Lift the pharynx with forceps and cut off the muscles that connect it to the body wall. Trace the connections between the **brain** and the **ventral nerve-cord** and look for nerves from the brain and from the collar-like **connectives** around the oesophagus. Determine the number and place of exit of the **nerves** arising from the ventral cord in the region just back of the "collar" and in the body at posterior end. Cut across the nerve-cord in the mid-body region and remove a bit by tearing out with a quick pull of the forceps. This piece may help you determine the number of nerves per segment.

**Exercise 8.** Make a diagram of the nervous system from a dorsal or a lateral view. Scale of 3 or 4.

## VI. TRANSVERSE SECTIONS

(a) Cut transverse sections of an alcoholic specimen about one segment in thickness, using a sharp pair of scissors. Study under water with the hand lens and make out the position of **gut, coelome, nephridia, septa, nerve-cord, blood vessels,** etc. Can you distinguish the different layers of the body and gut walls? Notice the **typhlosole,** a fold of the dorsal wall of the gut. How may it be of importance in digestion and absorption?

**Exercise 9.** Make a drawing, about three inches across, which will show the relative position and thickness of the various parts.

(b) Study the permanently mounted sections of this same region. Examine first with the hand lens and then with the low power to make out the appearance of the parts in such a

section. Then study the cellular structure of each part with the high power.

(c) In the body wall there are four cellular layers. 1. The **epidermis,** composed of short columnar epithelial cells, some of which are **gland cells,** in various stages of activity. Covering the outer surface of all these cells is a continuous membrane, the **cuticle,** of non-cellular nature and produced as a secretion from the epidermis. 2. A layer of **circular muscles,** here cut lengthwise. Are there **nuclei** and **blood vessels** among these? 3. The thickest layer is a series of **longitudinal muscles,** cut transversely, and so arranged as to have a feather-like appearance when the groups of fibres are seen in this plane. 4. The innermost layer is the lining of the body cavity, or **peritoneum,** which may show cell outlines and **nuclei** in favorable sections.

(d) In the intestine there are three chief layers. 1. The innermost of elongated, very narrow cells, the **mucous membrane.** 2. The outermost, a granular mass that may be resolved into a layer of large cells, the **chlorogogue cells.** 3. The middle, a narrow band of **muscle fibres,** both **longitudinal** and **circular.** Are there any blood vessels? Any cilia? The typhlosole will again be recognized. What is the condition of these three layers in this region of the gut? Blood vessels, nephridia and septa cut at various angles may require some careful study in your particular section. Try to understand these parts and why they appear as they do in any one section.

(e) Examine the structures which appear in the coelome and interpret them in terms of the organs seen in this region during your dissection.

**Exercise 10.** Draw on a large scale a narrow section of the body wall to show a small portion of each of the foregoing and on the same page a similar portion of the intestine to be located in the right position relative to the body wall. Add anything made out in the coelom.

(f) Study the nerve cord in the above section and note the outer layer containing **muscle fibres,** the three large clear

dorsal areas or **"giant fibres,"** the **fibres** of the main mass, and the position and structure of the **ganglion cells.** Understand from lectures or text-book the nature of the connections between the cells of the nervous system.

**Exercise 11.** Make a drawing of the nerve-cord in section, about 2 by 3 inches.

## THE CRAYFISH.

PHYLUM, ARTHROPODA. CLASS, CRUSTACEA

### I. LOCAL SPECIES

Two species of the genus **Cambarus** are commonly found in this vicinity. **C. virilis** is the more abundant and is the form seen in such numbers in the streams and ponds about Columbia throughout the open months. **C. gracilis,** one of the burrowing crayfish, is seldom found in the open water except during February and March, when the females come from their burrows along the banks of creeks and ponds and may be seen in in the water with their young. By the latter part of March these have returned to their burrows, but the young may still be found in the open water until the end of May. The presence of **C. gracilis** during the remainder of the year is however much in evidence from the **"chimneys"** which the animal builds around the air holes of its burrows. These openings are very common in moist ground and are often found at a surprising distance away from any body of water.

### II. THE LIVING CRAYFISH

(a) Watch live crayfish in shallow pans or aquaria and study their manner of swimming and walking. How sensitive are they to touch? By passing your hand across a short distance above the specimen, see if it shows any sign of an

acute sense of sight. Try this again with a specimen out of water. Place a specimen on its back in the water or on a table and determine the acuteness of its sense of equilibrium.

(b) Put some carmine in the water and see if you can detect any currents flowing in a definite direction in the vicinity of an animal when it remains quiet for a short time. Selecting a small individual, or one having a very clean shell, look on the outside of the body just above the great claw and and in line with the eye and see if you can detect a flickering motion, as though something were moving beneath the semi-transparent shell. Recall this observation later when you come to study the gill chamber. Keep a specimen out of water for a few minutes and note the bubbles which come from the front part of the body when it is again placed in the water. Do they come from a definite place on the body? You should be able to give an intelligent explanation of the facts noted in this paragraph after you have studied the gill chambers of the animal as outlined in section IV of these notes.

(c) Observe crayfish as they remain undisturbed in aquaria containing stones or other objects. Can you tell what determines the particular places which the animals occupy. Drop small pieces of fresh meat into these jars and watch the result. In some of the jars several small fish may be placed and the result watched at this and the next laboratory period. Observe crayfish in the large tanks or in nature and note any habits of concealment and also their mode of swimming.

## III. GENERAL EXTERNAL FEATURES

(a) For this study a good sized specimen may be killed with some anaesthetic or a preserved specimen may be used. Compare the anterior and posterior ends, the dorsal and ventral and right and left sides. Is there any departure from a strict bilateral symmetry? Examine the pairs of **limbs** from the posterior to the anterior end. Is the whole animal covered by a dense **shell?** What parts are moveable? How many divisions in the posterior part, so-called **abdomen?**

**Exercise 1.** After looking carefully at the proportions of the body, draw on a scale of 1 the outline of an ideal cross section through the abdomen to show the shape of the dorsal and ventral parts of the shell and the shape and attachment of the paired limbs. Do not actually cut across the specimen, but make the figure as it would appear if cut across.

(b) Find the **mouth** and **anus.** Note the round openings on the bases of the longest feelers (**second antennae**), they are the openings of the kidney-like **green glands.** Find on the dorsal face of the small first antennae the clear flat areas which mark the position of the **lithocysts** or organs of equilibration. Examine the bases of the walking legs for openings; they are found on the last pair in the male and on the second from the last pair in the female. In the male of **C. virilis** the two first pairs of abdominal appendages are modified to form, when pressed together, a **copulatory organ** along which the sperms pass after leaving the male openings. What is the condition of the corresponding appendages of the female? Place a male and a female side by side and note the difference when they are viewed from the dorsal side. The body of the crayfish consists of three regions, **head, thorax** and **abdomen.** Can you find anything on the dorsal side which might indicate the line of division between the head and thorax?

## IV. THE GILLS AND GILL-CHAMBERS

(a) Note how the shell, **carapace,** extends from the back down over the bases of the walking legs. Lift up the free ventral edge and see the spongy mass formed by the **gills** or **branchiae.** Taking care not to injure the gills and using your strong scissors, remove the overhanging shell from the left side, thus exposing the full extent of the **gill cavity,** but do not cut too far dorsally and injure the organs on that side of the body. Cut off the walking legs and large claw, **chela,** of this side a short distance from their insertion. Place the specimen under water in a dissecting dish and by floating up and carefully parting the mass of the gills get an idea of what a single

gill is like and where it is attached to the body. Move the stumps of the legs and see how the outer gills are related to them. How many of these outer gills are there? To what appendages are they attached? What effect do you think the animal's walking would have upon respiration? What structure do you find at the place where you saw the flickering movement under the shell of the live specimen? Back of this **"bailer"** is another, more delicate, blade which you will identify later as the **epipodite** of the first maxilliped.

(b) Put together all you know about the gill cavity and its contents, the water currents you have seen in the vicinity of a quiet animal and explain how the gills are always bathed with a constantly changing supply of water.

(c) These outer gills are called **podobranchs.** Note the significance of the name. Keeping the specimen entirely under water and lifting the podobranchs one at a time to be sure you do not destroy any of the smaller gills which lie close beneath, remove all of the podobranchs by cutting them off close to their attachment. Cut one across the middle with scissors and examine the section under water with a hand lens. You should see the **incurrent** and **excurrent blood vessels** cut across where they run close together.

(d) The inner layer of gills is now exposed. Are they attached to the feet? There are five pairs and a single one in front. Opposite which of the appendages are these gills located? They are called the **arthrobranchs** (joint gills.) Note again the significance of the name.

(e) In the lobster there is another layer of four gills lying beneath the arthrobranchs. Because they are attached higher up and on the sides of the body these last are termed the **pleurobranchs** (side gills). The common European crayfish from which the descriptions in most text-books are taken possesses a single pleurobranch, but in the adult of our **C. virilis** even this has disappeared. Examine museum specimens of the lobster dissected to show the three kinds of gills.

**Exercise 2.** Make an outline of the cephalothorax in a side view, on a scale of 2. Show the stumps of the appendages and

the places from which podobranchs have been removed. Put in all the arthrobranchs and show also the "bailer" and the epipodite above noted. Indicate the course of the water current by arrows.

(f) Examine specimens macerated in caustic potash and notice the delicate chitinous covering of the gills which has survived the maceration. Are the gills inside or outside the body? In answering this question imagine how they would look in a cross section of the animal in the thoracic region of the body.

## V. INTERNAL ANATOMY

(a) Using a freshly killed specimen, cut with large scissors along the dorso-lateral surface on either side of the cephalothorax, taking care not to injure any of the organs lying immediately beneath the skeleton. Remove this dorsal part of the skeleton from the posterior margin of the thorax to a point just back of the eyes. Place the specimen in a dissecting dish and having it entirely covered with water identify the following: The tops of the **gills,** which are exposed where you have cut into the **gill cavity,** are seen on either side. The **heart** which may be still beating lies between these on the mid-line in a cavity known as the **pericardium.** It is soft and spongy in its consistency and you should be able to distinguish the paired openings, **ostia,** which lie upon its dorsal surface. How many are there? The **gastric mill** or **gizzard** lies well to the front and is roughly triangular. Note its thin and delicate walls and the two transverse bars of harder material, by which its walls are strengthened. When the specimen is intact muscles pass from each of these bars, or **sclerites,** to the inner face of the dorsal skeleton. Find the remains of these muscles still attached to the shell which you removed, and also to the posterior sclerites. If the carapace has not been removed too far forward you should be able to see the muscles arising from the anterior sclerite and attached to the inner face of the shell just behind and between the eyes. These muscles form a part

of the complex system by which the grinding of the gastric mill is brought about. Passing through the pericardium are large muscles which diverge as they pass forward. If these pull on their forward ends as the fixed point, what movement will they bring about in the abdomen? You can answer this if you understand how the segments of the abdomen are articulated to one another. For this see specimens macerated in caustic potash. The appearance of the region between the heart and gizzard differs with the sex and sexual maturity of the specimen. In a female, with well developed **ovaries,** these latter organs are seen as a bi-lobed mass in front and a median mass behind the heart. In a male the **testes** are less conspicuous, but have the same general "Y" shape. In specimens which are immature or which have recently shed their eggs or sperms the organs are quite inconspicuous and need not be noted for the present. The **digestive gland** which is of a yellowish green color in a freshly killed specimen will be easily made out, but in specimens with large ovaries it may be crowded almost out of sight and only found by pressing aside the latter organs.

(b) Cut off the tops of the gills, sever the extensor muscles of the abdomen at the level of the heart, cut back along either side of the abdomen as far as the telson and remove the dorsal skeleton of this region. The **abdominal extensors** will be found as two thin bands of muscle lying close under the skeleton. They may be taken off with the skeleton, though you should be careful not to tear away anything else. The **intestine** will now be seen in the abdominal region along the mid-line. Beneath and to the sides of the intestine are masses of muscle, which by their combined action flex the abdomen. Compare the bulk of these flexors with that of the extensors. Why should there be such a difference in the size and hence the power of these muscles? Lying on top of the intestine you will perhaps make out a very small transparent thread, the **dorsal abdominal blood vessel.** At the anterior end of the abdomen the median portion of the reproductive organs may be found or, if these are immature, the posterior ends of the digestive glands.

**Exercise 3.** Make an outline on a scale of 2 or 3, of the cephalothorax and abodmen. Put into this the organs as they now lie in place.

(c) Remove the heart and look for ostia on its ventral surface. Note the "Y" shape of the reproductive organs and find their **ducts** leading to the external openings before noted. Remove the reproductive organs, being careful not to injure the digestive gland or the intestine. Trim off more of the gills and pull away the portions of the abdominal extensors which remain in the thorax. Make out the connection of the gizzard with the intestine and the antero-posterior extent of the digestive glands. Cut in from one side and find the **oesophagus;** it is very short and can be best located by noting again the position of the mouth. Trace the intestine to its posterior end, cut off close to the anus and carefully free it up to its union with the gizzard, also free the digestive glands. Cut across the oesophagus and remove the entire digestive tract and its appended glands in one piece. Float out in water, and cut off the left digestive gland close to the tract. Note the region between the gastric mill and the intestine. Open the gizzard along the ventral mid-line, find the teeth, work them together and see how they grind.

**Exercises 4.** Draw a side view from the left, showing the tract and the right gland in position and the place where the left one opens into the tract.

(d) To study the Nervous System, carefully remove all the muscles and viscera from the abdomen and the **ventral nerve cord** will then be seen lying on the mid-ventral line. Notice the **ganglia.** How many do you count? Notice the **lateral nerves.** How many of these? In the cephalothorax the nerve cord is concealed beneath transverse ridges of the ventral wall of the shell. Cut these with heavy scissors and expose the nerve cord, beginning at the hinder end of the cephalothorax and working forward. How many **thoracic ganglia** do you find? Just back of the oesophagus is the large **sub-oesophageal ganglion,** which is connected with the brain by two **connectives** passing around the oesophagus. The

**brain** or **supra-oesophageal ganglion** is just behind the eyes. Find the nerves passing from the brain to the eyes and to the two pairs of antennae.

**Exercise 5.** Draw a figure of the nervous system thus exposed, showing accurately the number of ganglia made out, the segments in which they lie and the number of lateral nerves.

(e) At the anterior end of the body, near their external openings already noted, find the **excretory organs** or **"green glands."** Show the position and shape of these by a dotted outline added to Exercise 3. The thin bladder and underlying **glandular portion** of the organ can be readily distinguished. Refer to text book for further details.

## VI. THE APPENDAGES

(a) For this work use either a fresh or a preserved specimen. In drawing take the appendages one at a time from the **right** side of the animal and **arrange each in such a way that when completed all your figures will have the same orientation.** This is very important for your correct understanding of the homologies between the various appendages. It is also important that the parts of each appendage drawn be completely labeled and that smaller ones be drawn on a scale of 2 or 3.

(b) There are all told 19 pairs of appendages. Beginning with the abdomen, count the number of pairs in this region of the body and compare them with the number of segments. The last pair of these is called the **uropods,** "tail-feet", the others the **pleopods** or **swimmerets.** Remove the right appendage of the fourth abdominal somite by cutting close to the body. A basal piece, the **protopodite,** bears two terminal pieces, an inner **endopodite** and an outer **expodite.** However markedly any of the other appendages may seem to differ from this plan of structure, all can be shown to be derived from this fundamental plan. The only exception is found in the case of the first antennae.

**Exercise 6.** Draw the appendage on a scale of 3 placing it with the end of attachment upward, the exopod to the right

and the endopod to the left. Use this same orientation in all your other drawings of appendages.

**Exercise 7.** Remove and draw on a scale of 3 the uropod of the right side. Label its parts and orient as in the last.

(c) Note again the difference in the two anterior pairs of abdominal appendages in the two sexes. A study of the embryology shows that they are all formed by the modification of the type above explained.

(d) The thorax has eight pairs of appendages as follows: Four pairs of walking legs or **pereiopods,** the great claws or **chelae** and three pairs farther forward which will be examined presently. Remove the right fourth pereiopod and the right chela being sure to get all of the seven and the six parts of which they respectively consist. In the pereiopod the two proximal parts represent a divided protopod while the remaining five are divisions of the endopod. In the embryo an exopod is present. The great claws are like the two anterior pairs of pereiopods save for the union of two of the divisions. Can you find where this has occurred? Note the simple modification by which the nipper is formed on the chela.

**Exercise 8.** Draw this pereiopod in the same orientation as your previous figures and show by a dotted outline the position the exopod would have if present.

(e) In front of the great claws are three pairs of appendages, known as the **maxillipeds** (jaw feet). The most posterior pair, or **third maxillipeds,** are large and easily recognizable. Before removal, the right hand member of this pair should be compared part by part with the walking leg just examined. It has the same parts except that an exopod is present and at one point two of the segments have fused to form a single one as in the chela. This third maxilliped is a very important appendage from the fact that it still has the fundamental plan and so can be compared with the simpler abdominal appendages, while the structure of its endopod shows how we may interpret the adult structure of the walking leg.

**Exercise 9.** Draw this appendage oriented in the same way as the others, on a scale of 3.

(f) Examine, without removing, the **second maxillipeds** which lie in front of the third. They will be found to have parts similar to the latter. They should be removed with care not to destroy the **first maxillipeds** which lie close in front of them. Identify, as before without removing, the parts of these first maxillipeds. There is a large **epipodite** which lies in the gill chamber just behind the bailer. Protruding toward the mid-line are two thin flaps which are an outgrowth from the protopod, and at about right angles to these are two other projections which are the exopod and endopod. Which is which?

**Exercise 10.** Remove the right one and draw on a large scale, orienting it properly.

(g) In front of the first maxillipeds are two pairs of **maxillae,** the parts of which should all be identified before the attempt is made to remove either one. The posterior or **second maxillae,** have a four cleft protopodite, a delicate endopod and an exopod which is fused with the epipodite so that it looks like a forward continuation of the latter. What is the function of the fused exopod and epipodite? Before this appendage is removed the parts of the **first maxilla** should be identified. This smallest of all the appendages consists of three parts, the endopod and a bi-lobed protopod. Which is which?

**Exercise 11.** Remove the right second maxilla and draw properly oriented and on a large scale.

**Exercise 12.** Remove and make a similar figure of the right first maxilla.

(h) The **mandibles** will now be exposed. Against their posterior surfaces are a pair of lobes which are not true appendages. Each mandible consists of a heavy basal portion on the median side of which is located the cutting edge which is shown by the embryology to be a development of the protopod and which is comparable to the more delicate median outgrowths on the first and second maxillae. The three-jointed **palp** which protrudes from the heavy basal piece has its proximal joint formed from the protopod and the other two from the endopod. The exopod is wanting in the adult. Where would it be if it were present?

**Exercise 13.** Remove and draw the mandible of the right side properly oriented and on a scale of 3.

(i) The **second antennae** will show, when examined in place on the specimen, the typical exopod, endopod and protopod and the opening of the green glands.

**Exercise 14.** Remove the right one of this pair, orient, and draw on a scale of 3 to show these points.

(j) The **first antennae,** or **antennules** as they are sometimes called, are the only ones which do not show a real division into the three fundamental parts, although their two terminal portions would at once suggest the endo- and exopod. Remove one and examine more carefully the region of the **lithocyst** or organ of equilibration.

## THE FRESH-WATER MUSSEL

PHYLUM, MOLLUSCA. CLASS, LAMELLIBRANCHIATA

### I. HABITAT, ACTIVITIES AND EXTERNAL FEATURES

(a) The fresh-water mussels or clams are represented in the Mississippi Valley by many genera and by species which are numbered in the hundreds. They occur most abundantly in running water, but some species are more common in ponds and sloughs. For the most part, the species resemble one another to such an extent in their general structure that the following notes may be used for any one of a number of forms likely to be used for laboratory work. If specimens are available, examine the shells of several representatives of a single genus and compare them with one another and with the species in another genus. Try by doing this to get a more concrete idea of what is meant by a **genus** and a **species.** Of late years the shells of these mussels have assumed a considerable com-

mercial importance in the manufacture of fresh-water pearl buttons and, as a by-product, of the lime grits used for chickens. There have thus come into existence common names for all the easily recognizable species a few of which are given below.

**Quadrula ebena,** the nigger head.
**Q. pustulosa,** the warty back.
**Q. metanevra,** the maple leaf.
**Lampsilis ligamentinus,** the mucket.
**L. anodontoides,** the yellow back.
**L. rectus,** the black sand shell.
**Symphynota complanata,** the hatchet back.

(b) Before adding the title to any of your figures, the species you have been dissecting should be identified, either by consulting a collection of named shells, or with the aid of an instructor.

(c) The points which can be observed by study of the living animals are better appreciated after one becomes familiar with a pair of shells. Examine such a pair. They are right and left. The **hinge** is dorsal, the gape the ventral side of the animal. **"Lines of growth"** mark the outer surface. These indicate periods of active, alternating with slower growth. What is the oldest part of the shell judging by these lines? This part is called the **umbo.** A line drawn through the umbo and at right angles to the long axis of the shell will divide it into distinctly unequal parts. In all species the smaller of these is anterior. Some species show bands of different color radiating from the umbos, others have protuberances or ridges of various sorts on the shell. Why are shells very commonly eroded at the umbos?

(d) Having thus oriented the shell, note other more detailed points. Has the hinge more than one layer? Examine the edge of a piece of broken shell showing three layers, the **periostracum** on the outside, the **prismatic layer,** and the **mother of pearl.** What is their relative thickness? Can you find any lines of growth which will give a clue as to how the shell grows in thickness? A demonstration of the prismatic layer will be found at the centre-table.

(e) Inside are **teeth** which lock tightly when the shells are together. What function may these have? Good sized markings indicate the places of insertion for the **anterior** and **posterior adductor muscles** which pull the valves together. Since there are no muscles which can pull the shells apart how might this be accomplished. Shells specially prepared to demonstrate this will be at the centre-table.

(f) There are other muscle scars considerably smaller, but easily recognizable. They mark the insertion of muscles which move the foot as will be seen later. They are the **posterior retractor** scar, above the posterior adductor; the **anterior retractor,** which is posterior to the anterior adductor; and the **protractor,** which is a short distance below the anterior retractor. Extending from one adductor to the other and parallel to the margin of the shell is a marking known as the **mantle line.** This is the so-called "water line," often seen in fresh water pearl buttons of the larger sizes. What can you make out at the edge of the shell regarding the three shell layers?

**Exercise 1.** Draw, on a scale of 1, the outer surface of the right valve with its dorsal margin toward top of page. Below this the inner surface of the left valve should be represented in the same orientation. In beginning the figure, put the right valve down on the paper and trace the two outlines about it. Show all the points of the foregoing section which can be represented. Care is necessary in properly representing the external lines of growth.

(g) Test for yourself, or by examining the results of a demonstration at the centre-table, determine the effect of acid upon the substance composing the shell and also the effect of a strong alkali like caustic potash. Of what is the shell composed? What reason is there for the fact that heavy shelled mussels are abundant only in regions of limestone rock?

(h) Living mussels should now be studied in large aquaria, or in individual dishes with enough sand on the bottom to allow the clams to bury themselves readily. **Lampsilis subrostratus,** a small pond form, is admirable for this purpose and may be examined in a finger bowl. Place the mussel on its

side and watch it begin burrowing. The fleshy organ which can be protruded from between the antero-ventral margins of the shells is the **foot.** How does the animal make its way down into the sand or move about? The fleshy membrane exposed between the slightly gaping valves is the **mantle.** In a specimen which lies undisturbed on its side or one which is embedded in the sand, can you see openings between the right and left sides of the mantle at the posterior end of the animal? Are there **papillae** along these openings or elsewhere along the mantle margin? Touch parts of this region very gently with a needle and determine its sensitiveness. Can you distinguish any difference in the degree of sensitiveness between the region of these two openings, or **siphons,** and the part of the mantle near the foot? In a quiet specimen, with the siphons well open, watch for currents of water in and out of the clam by way of the siphons. The existence of currents may be demonstrated by dropping powdered carmine into the water near the siphons with a clean pipette, or there may be enough silt in the water to show the same thing. There is a constant, though gentle, current in one siphon and out the other. Which is the **inhalent** and which the **exhalent siphon?** When the mantle edge is strongly stimulated with the needle note how the shells quickly close driving water out through both siphons. By examining a number of mussels which have been left undisturbed for some days in an aquarium with sand, determine the normal positions of the animals in their life on the bottom. The water currents are of great importance to the animal since in addition to the supply of water for respiration they bring the entire food supply which consists of the micro-organisms living on and near the bottom. How the animal acts like a sieve which strains out the water and holds the organisms will be explained when the internal organs are examined.

(i) If the dish can be placed in sunlight, test the sensitiveness of the siphonal regions to light by casting a strong shadow over this end of a well expanded specimen.

**Exercise 2.** Such points in the foregoing as can be well described in writing should be properly incorporated in your laboratory book.

## II. EXTERNAL STRUCTURE*

(a) A specimen preserved in formalin, or one just killed should be used for this. As the removal of the shell is not easy for a beginner, you should have the aid of an instructor for this preliminary step. Remove the **right** valve and study the mussel from this right side as it lies in the other valve. You will then have your specimen in the same orientation as the drawings of the shell. When the shell has been removed, note how the mantle everywhere conforms to its inner surface. There will be no breaks in the mantle unless it has been mutilated in the removal of the shell. Find the ends of the following muscles the scars of which you have already seen upon the shell, **anterior** and **posterior adductors, anterior** and **posterior retractors** and the **protractor.** Find the line on the mantle which corresponds to the **mantle line** on the shell. The following internal organs can be more or less definitely recognized according to the species or the method by which the specimen has been prepared: **digestive gland, kidney, Keber's organ,** and **pericardium** containing the heart. By consulting a chart or blackboard diagram, understand their position even if you are not able to locate them at this time in your own specimen.

**Exercise 3.** Make a figure of the mussel, as it thus lies in the left valve of its shell and seen from the right. The removed valve may be wiped dry and the first outline of the drawing laid down by tracing around it.

(b) The space enclosed between the right and left halves of the mantle and in which the foot lies, is the **mantle cavity.** Without tearing or cutting, find how the incurrent siphon communicates with this and by lifting up the mantle edge get an idea of the foot and the four plate-like **gills,** which extend from

*The features of the mantle surface and the organs in the mantle cavity are really a part of the external surface of the animal.

the sides and top of the foot to the region of the siphons. Look carefully and see the line along which the outer surface of the outer gill and the inner surface of the mantle meet. Find the **palps,** a pair of organs on either side of the foot posterior to the anterior adductor muscles and note their attachment to the inner surface of the mantle. Remove now the right half of the mantle by cutting from the middle of the incurrent siphon along a line about one-fourth inch below and parallel to the place where the gills and inner face of the mantle meet. At the region of the palps care should be taken to leave these organs intact. Continue the cut just below the anterior adductor muscle and thus expose the organs of the mantle cavity. Trim off to a neat outline the cut edge of the mantle, without injuring gills or palps.

**Exercise 4.** Beginning with an outline made by tracing with the right valve, construct a figure to show all of the above organs, omitting for the present the structure of the region above the cut edge of the mantle and the outline of the cut edge itself.

(c) The structure and function of the organs concerned in the water currents should now be studied. By looking into the uninjured ex-current siphon the **intestine** ending in the **anus** will be seen on the posterior face of the adductor muscle. Extending beneath this adductor anteriorly, is a cavity into which a bristle may be thrust for a considerable distance. Being careful not to cut too deep, make an incision at the top of the outer gill near the middle of its length and expose a cavity running along the top of this gill. By gently probing with the bristle, explore this cavity forward and back. How does it end in either direction? With the bristle thrust in as a guide, cut in either direction and expose this **supra-branchial** cavity from the anterior end of the gill to its opening into the **cloaca,** as the region just within the excurrent siphon is called. Trim away the tissue on each side of the cut so that the whole diameter of this supra-branchial cavity is readily seen. The upper line of the cut edge should be made to pass along the lower margin of the posterior adductor and end above the anus,

the lower cut edge should pass out to where the two siphonal openings meet. The floor of this cavity is cut by a series of transverse partitions **inter-lamellar junctions** separating cavities, the **water-tubes** of the gills. If the gill is not too much shrunken you can pass a bristle down any of these water-tubes to the ventral edge of the gill where it ends blindly. Making a clean cut with the scissors at right angles to the water-tubes, remove a piece of the gill and examine the cut edge under water with a hand lens. The water-tubes and inter-lamellar junctions will be seen cut transversely. It is in these water-tubes that the young of the clam begin their development and you may find the gill distended with embryos. By looking forward from beneath the posterior adductor and gently exploring with a bristle, you can see that the inner gill of this side and the two gills on the other have each a supra-branchial cavity and water-tubes as in the gill just examined. How would the four supra-branchial cavities be connected with the cloaca if looked at from the dorsal side? Sketch a diagram of this system of cavities as it would appear if viewed dorsally.

(d) You should now be able to understand how the water current, from which the clam gets its food and oxygen and by which its carbon dioxide and other wastes are carried out, flows through the gills. Coming in by way of the incurrent siphon the water freely bathes the organs of the mantle cavity. From here it passes through microscopic openings, the **ostia**, which lead from both inner and outer surfaces of all the gills into the water-tubes. Passing upwards in the water-tubes, it emerges in the supra-branchial cavities and passing backward in these it reaches the outside through the cloaca and exhalent siphon. The water which passes out has been strained of its micro-organisms which are too large to pass through the ostia of the gills. These organisms upon coming in contact with the slimy surfaces of the gills, foot or mantle are entangled in the mucus and carried by cilia along definite lines which finally bring them between the palps and to the **mouth** which will be found anteriorly below the adductor and between the continuations of the inner and outer palps.

(e) Examine under the microscope a bit of tissue cut from the gill of a living clam and one from the mantle and make out the cilia on these surfaces. The ostia will be demonstrated in stained sections of the gill taken in the same plane as the rough sections previously made with scissors. Place carmine or pieces of cork upon the gills or upon various parts of the mantle surface of a living specimen and note the result.

**Exercise 4 (continued).** Add to your previous drawing these details of the supra-branchial and cloacal cavities, taking care to show the cut edges where they occur. Put in arrows to show the course of the water currents.

(f) Why are we justified in speaking of the mantle cavity as a part of the external surface of the animal?

## III. INTERNAL STRUCTURE

(a) The mantle, supra-branchial and cloacal cavities are really portions of the outer surface of the mussel and only in what follows are we dealing with organs which are internal in the same sense as the viscera of a frog or other familiar animal.

(b) In the region above the gills and in front of the posterior adductor is the **pericardium.** Make a small incision in its wall and lifting with forceps cut the wall away exposing the **heart,** consisting of a single median **ventricle** wrapped about the **intestine** which traverses the pericardium, and of delicate right and left **auricles** which lead from the sides of the pericardial cavity. These last can probably be better seen if they are floated out under water. The dark colored **kidney** will be seen underlying the pericardium.

**Exercise 4 (concluded.)** Add to your figure the pericardium as thus exposed and its contained organs. Show clearly the cut edge of its wall.

(c) Lift up the outer gill and remove by cutting away the inner wall of its supra-branchial cavity. Locate the supra-branchial cavity of the inner gill and cut into this to expose its full length. The dark color of the kidney will probably show through the wall of this latter cavity, and well toward

the anterior end there will be found against this dark area two small openings. They are not as easy to find in some species as in others but can usually be located. The dorsal one of these openings is the **external opening of the kidney** of this side, the ventral is the opening of the reproductive gland or **genital pore.** On the other side of the foot are similar openings for the left side of the body. Note that the opening of the kidney is thus into a cavity from which the water flows immediately to the outside. The eggs upon leaving the genital pore are fertilized by sperm which have been shed to the outside water from an individual of the opposite sex and entered the supra-branchial cavity of the female. After being fertilized the eggs fall into the water tubes and there develop as far as the larval stage, known as the **glochidium.**

(d) Begin at the anal end and dissect the intestine entirely free from its union with the upper surface of the posterior adductor muscles. Turn the specimen up on the ventral edge of its shell so you can look into the pericardium from above and by taking hold of the intestine and turning it over anteriorly expose the extreme anterior end of the pericardium. Careful probing with a fine headed bristle should reveal the opening from the pericardium into the kidney. Push the bristle as far back as it will go and thrust another finely tipped bristle through the external opening of the kidney pushing it back also. Cutting into the substance of the kidney will now reveal the fact that one bristle lies in an upper and thin-walled, the other in a dark colored and thick-walled cavity. Near the posterior adductor these upper and lower limbs of the kidney unite.

(e) With the handle of a scalpel scrape the tissue of the kidney away from the top of the foot and note the two posterior retractors, the ends of which have been previously observed. Still using the scalpel handle, free these from the adductor muscle and the left one where it attaches to the shell. Can you see why they are called retractors of the foot?

(f) Leaving the intestine intact and attached to the foot and **visceral mass** (the term applied to the part of the body which lies above the foot and is softer and less muscular than

that organ), continue with the handle of the scalpel and break away the attachment of the visceral mass to the shell in the region of the teeth, being careful not to injure the mouth. Remove this part of the body from the shell leaving the anterior adductor behind.

(g) Before discarding the left valve with the posterior adductor attached, look on the ventral surface of this muscle for a yellowish body which is the fused right and left **visceral ganglia** of the nervous system. Look for nerves extending out from this. The pair of **cerebral ganglia** will probably also be seen upon the ventro-posterior face of the anterior adductor mussel. Indicate the position of these ganglia upon your general drawing.

(h) Locate the anterior retractors and the protractors of the foot and see why they are so called. Remove the palps and any remains of mantle or gills from each side of the visceral mass and then with a sharp scalpel split this mass and the foot as nearly into right and left halves as possible, leaving the free end of the intestine attached to the left half. Examine the cut surface of the left half under water and pinned down in a pan. The visceral mass will be seen to be composed of a pasty mass, made up largely of the reproductive gland, in which the coils of the digestive tract are embedded. Follow any of these canals which are cut by the section. The flattened oesophagus leads upwards to an enlargement of the **stomach** into which the right and left halves of the **digestive gland** open. The following out accurately of the digestive tract in any species is a difficult piece of dissection for which a second specimen will be supplied if you have additional time. The course of the tract should be examined in text-book or chart figures.

(i) In the soft tissue just above the upper margin of the foot and a little distance below the mouth, you will find by gentle scraping, if they are not already exposed, the pair of **pedal ganglia.** They are yellowish in color and of firmer texture than the tissue in which they are embedded. Nerves will be found running out from them. Show in your general drawing the position of these within the substance of the vis-

ceral mass. Understand from lectures, text-book or charts, how the three pairs of ganglia, you have noted, are united by paired connectives and what parts of the body their nerves supply. If you have time, a specimen will be supplied for a special dissection of the ganglia and their connectives.

## IV. THE EMBRYOLOGY AND PARASITISM OF THE MUSSEL

(a) At certain periods of the year the gills of the mussel are found distended with the **glochidia** which have been previously referred to. Examine in a watch glass of water some of these glochidia just removed from a freshly opened mussel. Note the two halves of the **shell,** the **adductor muscle** between them and certain fine projections, the **sensory** hairs on the inner surface. Are there **hooks** at any point on the shell? Beyond the valves of the shell none of the organs of the adult are visible. These embryos are shed from the mussel, by way of the out-going water current, and scattered upon the bottom where they must come in contact with the fins or gills of a fish and fasten themselves there in order to continue their development. After leaving the fish they have developed all the organs of the adult in miniature and can begin life on the bottom. Watch the glochidia for any movements and record nature of same.

**Exercise 5.** Draw the glochidium on a large scale, as it appears gaping open and also from a lateral view when closed. The closure of all the specimens can be easily effected by adding a few drops of waste alcohol or of methyl-green.

(b) Take now a considerable number of living glochidia in a finger bowl of clean water and put into this one or two small fish. Watch how and where the glochidia attach. If the fish do not keep the water sufficiently agitated to prevent the glochidia settling to the bottom it must be stirred gently. After five or ten minutes take out the fish and put one into an aquarium. Kill the other without pressing upon the gills or fins and pin it ventral side up in a pan of water. Remove gills,

fins and tail and examine with microscope in a watch glass. How and where are the glochidia attached?

**Exercise 6.** Draw one or more glochidia attached to the gill or fin, and on such a scale as to make the larvae about one-half inch in diameter.

(c) Make an estimate of how many glochidia there are on this one fish, of how many were produced by the single mussel.

(d) The fish which were set aside after infection should be examined at the next laboratory period and the condition of the glochidia with reference to the tissue of the fish determined; or fish infected 24 to 48 hours previously may be provided for examination on the same day as the foregoing. Record the condition of these glochidia and if you have time make figures.

## V. TRANSVERSE SECTIONS

(a) For a review of many points brought out by the foregoing study the examination of sections, cut through a specimen from which the shell has been removed, is valuable. In sections from the region of the heart, which are perhaps more instructive than any others, the following structures should be made out and compared with the conceptions and figures already obtained; **mantle, foot, gills, supra-branchial, mantle** and **pericardial cavities, kidney, Keber's organ, ventricle, auricles** and **intestine.** How is the shell related to the whole? How does the water pass from the mantle cavity to the supra-branchial cavities? Where are the water-tubes?

**Exercise 7.** Draw such a section on a large scale labeling all the parts and showing the course of the water by arrows.

## VI. SPECIAL DISSECTIONS

(a) A second specimen may be used for a review of the structures previously dissected and the more complete demonstration of the nervous and digestive systems. Remove the shell and mantle of such a specimen, examining again any

points not previously made clear. Locate the **cerebral** and **visceral ganglia** without cutting anything but the mantle. By careful dissection, follow one of the two nerves, which may be seen leading anteriorly from the visceral ganglia, to its union with the cerebral ganglia. From these latter, three pairs of nerves arise; the pair of **cerebro-visceral connectives** just dissected, a pair of **mantle nerves,** and the **cerebro-pedal connectives.** Follow one of the last to its union with the **pedal ganglia** and determine the number of nerves arising from the latter.

**Exercise 8.** Make a figure of the entire nervous system.

(b) This same specimen may be used for a dissection of the digestive tract. To do this remove the entire animal (including the two adductors) from its shell and pin out under water. Begin at the mouth and dissect out the tract to show, **oesophagus, stomach,** openings of **digestive glands** and coils of the **intestine.** These latter must be exposed by removing the side of the visceral mass which is uppermost and following with care each part of the intestine as it is found.

**Exercise 9.** Make a figure of the entire digestive tract.

## CYTOLOGY

### I. MITOSIS

The finer details of cell division must, of course, be studied with the very highest magnifications. The more general features may be examined with the high powers ordinarily used in a course of this nature. **Mitotic** or **indirect cell division** appears to be the common method by which cells divide. The **amitotic** or **direct** mode of division seems to be of less importance and its significance is still a matter of doubt. For the study outlined below, sections of growing onion root tips or the epithelium of salamander larvae may be used.

(a) Examine the sections with low power to understand the relation of the parts, then with highest powers look for cells in different stages of division showing the chromatic material in the form of **chromosomes.** Examine chart or text-book diagrams of mitosis and determine which phase of the process is represented by each cell found in division. Determine, if possible, **the number of chromosomes** in each cell.

**Exercise 1.** Construct six cell outlines for figures showing consecutive stages. Then put in the nucleus as you find good examples of the several steps in the process. Have the series in order when completed, but do not try to find them in this order. Rather, take representative cells as found in searching over the slide and draw into their proper place in the series.

(b) The following terms have come into use for designating the stages in cell division:

**Prophase.** The division and migration of the **centrosome** and formation of the **spindle,** the assumption by the **chromatin** of thread-like aggregates which segment into **chromosomes** and the arrangement of the chromosomes into an **equatorial plate.**

**Metaphase.** The lengthwise splitting of the chromosomes.

**Anaphase.** The divergence of the chromosomes into the two daughter groups, and the division of the **cytoplasm.**

**Telophase.** The appearance of a **nuclear membrane** in each daughter cell and the reconstruction of the nucleus to its typical **resting condition.**

(c) See that all the structures indicated above are properly labeled. Test your understanding of the spacial relations of parts by seeing whether you can readily interpret sections cut at irregular angles.

## II. MATURATION

(a) Demonstrations may be examined showing: (1) **polar bodies** in superficial view and (2) the **reduction divisions** of the **oocytes** and **spermatocytes** in sections. Understand the relation of this process and of fertilization to the number of chromosomes and its universal occurrence.

(b) Demonstrations of the accessory or **"x" chromosome** may also be shown. What relation has this chromosome to sex? What happens to this chromosome in the reduction division? In fertilization? Do you think we can properly speak of this as a **sex determiner?**

### III. FERTILIZATION

(a) Examine demonstrations showing the **entrance of the sperm** and the **conjugation** of the male and female **pronuclei** to form the **cleavage nucleus.**

## CLEAVAGE AND GASTRULATION OF ECHINODERMS

### THE EGG OF ASTERIAS OR ARBACIA.

The eggs and spermatozoa of many marine animals are laid directly into the water where they meet in fertilization. Such eggs are usually small, having but little yolk. They develop rapidly into feeding larvae, which swim for a time, and then take up the life of the parent upon the bottom. Because of the ease with which they can be provided with their normal environment, these eggs are particularly favorable for experimental studies and have become classic examples in the study of fertilization, artificial parthenogenesis, cleavage, and the like.

(a) Examine stained material permanently mounted or in the clearing fluid, showing **cleavage, blastula** and **gastrula** stages in the egg of the starfish or sea-urchin. Note the egg membrane, sometimes showing the heads of many spermatozoa which failed to enter. The **two-, four-, eight-cell** and later **cleavage stages** on to the **blastula** or hollow sphere stage will be recognized. Find stages of the blastula showing the ingression of **mesenchyme** cells at one pole. Is the wall of the

blastula of uniform thickness? Can you tell from the first the region that will **invaginate** to form the next stage, the **gastrula,** in which the primitive gut cavity or **archenteron** is formed. Its opening is the **blastopore.** The invagination is termed **gastrulation.** The **germ-layers, ectoderm** and **endoderm** have now been formed. The mesenchyme cells noted above and other cells which arise from the blind end of the archenteron constitute the **mesoderm.**

**Exercise 1.** Make a series of outline figures illustrating the foregoing.

(b) The type of cleavage here represented is designated as "total" or **holoblastic** and **alecithal** (without yolk). Compare later with condition in frog and chick. The blastopore becomes the **anus** of the larva. The **mouth** is formed by an invagination, the **stomodaeum,** which unites with the blind end of the archenteron. Examine demonstrations. A larva which is strikingly bilateral results and from this, by a curious **metamorphosis,** the radially symmetrical adult is formed. The existence of such a larva constitutes the main evidence for the belief that the present radially symmetrical echinoderms have descended from bilaterally symmetrical ancestors. Compare with the inferences drawn from the existence of fish-like stages in frog and chick.

## ONTOGENY OF THE AMPHIBIA

### I. BREEDING HABITS, ETC.

(a) If possible go out to collect frogs' eggs, examine the places where frogs and salamanders lay and make out what you can regarding their activities during the breeding season. Several of the species common about Columbia may deposit the eggs in February, or early in March, others at some time later in the spring, or even in early summer.

(b) Take home a mass of eggs you have yourself collected or have obtained from the laboratory. Place in a shallow dish or basin and keep in a light place, not exposed to direct sunlight for much of the day. Record the stage of the eggs when obtained and note their progress from day to day. Preserve your notes in the form of a written report to be handed in later. The influence of temperature upon the rate of development can be tested by placing part of the eggs out of doors on the cool north side of a building and comparing them each day with those having sun and the warmth of indoors. With proper care they may be kept until the tadpoles have completed their metamorphosis. At no time should the water in the dish be allowed to become too low from evaporation or to become foul from the growth of bacteria. Green water plants will be beneficial unless growing in too dense masses. When the tadpoles are fully formed, small bits of bread may be crumbed up and put in the dish. Too much of this will, however, foul the water and hence care must be used.

(c) Examine the living frogs and salamanders on exhibition in the laboratory and find out the species to which the eggs you are studying belong.

## II. THE UNFERTILIZED EGG, OR OVUM

(a) Examine in a watch glass of water a small mass of eggs from the ovary of a frog preserved in formalin. Look for small eggs among the larger ones of the present season. Some of these will show, when examined under the low power, the **nucleus** with its **nucleolus** and a small body of **cytoplasm.** Further study with proper material would show that the comparatively large eggs are **single cells** in which a great deal of nutrient **yolk** has been accumulated in the cytoplasm. The egg in these stages before fertilization is termed the **ovum.**

**Exercise 1.** Draw one of these small ova to show its parts as a cell.

(b) Permanently mounted sections of the ovary of a young frog may also be used to demonstrate the cellular structure of

the ovum. These will show ova of various sizes surrounded by the nuclei of smaller cells and the **nucleus, nucleolus** and **cytoplasm.**

(c) Remove a living egg from the ovary of a female frog and crush under a cover slip. What can you make of the structure when examined with the compound microscope?

## III. THE SPERMATOZOON

(a) Cut into a piece of testis and examine the milky contents in normal salt solution. Under the high power look for motile bodies, the **spermatozoa.** Each has a very long tail, not at first observed. Various nucleated cells may be found in the testes. Some of these can be recognized as stages in the formation of the sperms. During the breeding season, ripe sperm may be found in the seminal vesicles. As in the case of the ovum, the spermatozoon is a single cell.

**Exercise 2.** Draw a ripe spermatozoon and any other cells which are clearly younger stages of the same.

## IV. THE EGG-LAYING, FERTILIZATION AND COPULATION

(a) Recall the exact structure of the male and female reproductive organs. The **jelly,** which is so conspicuous a feature of the masses of eggs seen in ponds, is a product of the oviducts. Fertilization occurs in the water as the eggs leave the cloaca of the female and while the animals are joined in copulation, or before the swelling of the jelly during the first few hours exposure to the water. Take several eggs in which fertilization is in progress and which have the jelly well swollen. Note the individual **envelopes** in which the eggs are embedded. What is the characteristic difference between the jelly of the frog and that of the salamander eggs? Note the distribution of the color on the surface of the egg. How does it compare with the general distribution of light and dark color on adult frogs, fishes, birds, etc., with which you are familiar? Which

is the heavier side of the egg? The centre of the pigmented area is called the **animal** pole, the opposite point on the sphere the **vegetative** pole of the egg. If specimens showing the polar bodies are available, examine these and show in your next drawing.

**Exercise 3.** Draw, (a) several eggs, on a scale of 2 or 3, showing their envelopes and the general mass of jelly, and (b) a single one from a side view 1 inch or more across to show distribution of pigment and the jelly envelopes. Label the poles.

## V. THE DEVELOPMENT OF THE FERTILIZED EGG

(a) Where the stages noted in the ensuing account are followed in preserved material, it will, of course, be impossible to observe the actual progress of the development here described. Careful reading will, however, make clear what points may be observed in preserved material and the method of description will enable the student to follow more vividly the changes which the preserved stages represent. **When it is desirable to remove the jelly from any of these stages in preserved material, it may be easily done by rolling the egg along on a piece of filter paper.**

### CLEAVAGE STAGES

(b) If the living eggs are available, begin with the **zygote** or **one-cell stage** and watch closely for the **first cleavage furrow,** a dark groove which appears first on the animal pole and, as it grows deeper, spreads over the vegetative portion until it encircles the sphere. Observe its exact position with reference to the polar axis. Record the time occupied in the above process by which the **two-cell stage** is produced.

**Exercise 4.** Draw side and top views to show the furrow in process of formation and as it appears when completed. The most satisfactory size will be a figure 1½ to 2 inches in diameter, a scale which should be continued in the subsequent drawings.

(c) A short period now ensues during which no external changes occur, but internally the nuclei are preparing for the next cell division.

(d) Watch now for the **second cleavage furrow.** Record the time of its first appearance and of its completion.

**Exercise 5.** Draw a top view of this furrow just appearing and a view from the vegetative pole when it is complete.

(e) Careful watching is necessary lest the **third cleavage furrow,** which is horizontal and just above the equator of the sphere, come in unobserved. Record time of its appearance as in foregoing. With the completion of the third furrow we have the **eight-cell stage** which has four smaller, deeply pigmented cells above and four larger, lighter colored cells below.

**Exercise 6.** Draw a side, or top view of this stage numbering the furrows.

(f) Understand that with each division of any one cell the nucleus also divides, so that in the two, four, eight and later cell stages and so on to the many celled adult organism, each cell possesses a nucleus descended through a longer or shorter series of divisions from the original nucleus of the one celled stage or zygote which was itself formed by the fusion of nuclear material from egg and spermatozoon. Hence, we reach the generalization that every cell of the adult animal may contain a nucleus descended one-half from the male and one-half from the female parent.

(g) The next cleavage consists of two vertical furrows which appear simultaneously at right angles to one another and cut each of the eight cells approximately in halves. The four upper cells often complete this division before the furrows have appeared in the lower hemisphere, thus making, with the eight smaller above and the four larger cells below, a **twelve-cell stage.**

**Exercise 7.** If this is observed, draw from a top or side view, to show the twelve-cell stage.

(h) With the division of the four lower cells, the **sixteen-cell stage** is produced.

**Exercise 8.** Draw this from a side view.

(i) By very careful observation, it can be determined that there next appears an approximately horizontal furrow parallel to the plane of the third cleavage. This divides the eight upper cells. A corresponding cleavage plane divides the eight lower cells, thus making a total of thirty-two. A **thirty two-cell stage** is, however, a theoretical rather than an actual occurrence, because of the fact previously noted that the cells about the upper pole divide faster than the corresponding yolk laden cells below. Again, very careful observation shows that there next follow two more approximately horizontal planes of division, one above and one below those last mentioned, making a **forty eight-cell stage,** but here as in the thirty two-, this exact number of cells is probably never present because of the way in which the lower cells lag behind the smaller upper ones. From the eight-cell stage on, the cleavage is not carried out with geometrical regularity as will readily appear in the study of (g) and (h) above.

(j) The term **mulberry stage** is often applied to these stages. More definitely, we will speak of it as an **early blastula stage,** the complete blastula being reached only by the continued subdivision of the constituent cells.

**Exercise 9.** Draw the early blastula stage from a side view.

## BLASTULA AND GASTRULA

(a) A characteristic feature of the blastula is the existence of an internal **cleavage** or **blastula cavity.** In the amphibian, this cavity appears at about the twelve to sixteen-cell stage and persists until it is obliterated by the gastrulation process. Take a preserved specimen in the early blastula stage and after removing the jelly by rolling on filter paper, hold gently between forceps, without crushing, and divide in halves by a vertical cut with a **sharp scalpel.** Examine with hand lens and note the **cleavage cavity** and cell outlines.

**Exercise 10.** Draw the cut surface to show such an early blastula in vertical section.

(b) If the eggs are followed hour by hour it will be seen that during the second day, at room temperature, the dark cells of the animal pole begin to encroach upon the surface area of the lower light colored ones. The exposed surface of the lower cells thus becomes diminished around its entire margin by downward over-growth of the dark upper cells. By turning the egg upside down there will be seen along part of the circumference of the diminishing area of exposed yolk-cells a crescentic depression which is the **blastopore.** Understand from your lectures the internal changes which are now taking place. The stage now reached is called the **gastrula.**

It can be turned upside down for observation by placing on a slide a pin bent to an acute angle putting the specimen in the desired position in the angle and adding a little water and a cover glass. Find the blastopore with a hand lens and with low power of microscope. Look for cell outlines in the dark and in the light areas and indicate in the following figure.

**Exercise 11.** Draw the gastrula stage as seen from beneath.

(c) At a later stage of the gastrula when the dark cells have still farther encroached upon the exposed area of the yolk we have the **yolk-plug stage.** This may be examined in the same way as the last or cut in halves as in (a) above and examined in section.

**Exercise 12.** Draw this yolk-plug stage. Understand from your lectures and the text-book the internal structure of the gastrula, i. e., its **ectoderm, endoderm, mesoderm** and **archenteron.** The yolk-plug finally disappears within the embryo, but the blastopore can still be distinguished as a minute pit which is to be shown in your next drawing.

## NEURAL FOLD STAGE*

(a) The first sign of this stage appears about the time that the yolk-plug is lost to view. The ectoderm thickens along

*The directions as far as this point have been designed either for the eggs of a frog or a salamander, In the sections which follow the notes have been written with special reference to the salamander **Amblystoma tigrinum,** but will serve almost equally well for the frog.

a certain area and as a result of this a ridge appears upon the upper portion of the still spherical embryo. This ridge may be regarded as a continuous structure having something the shape of an hour glass, but it is customary to speak of the right and left portions as the two **neural folds.** Find the **blastopore** which now lies at one end of the area enclosed by these folds. With the appearance of the neural folds, the bilateral symmetry which characterizes the outside of the adult becomes obvious externally. Although this stage is not greatly changed from the spherical condition immediately preceding it, we can now clearly recognize the axes of the adult body as follows: The region between the neural folds is on the dorsal mid-line. The opposite surface is of course the ventral. The end where the folds are most widely separated represents the anterior, the end where the blastopore is located the posterior region of the future adult.

**Exercise 13.** Draw a neural fold stage from a top view as it normally lies within the jelly. Make outline of the jelly still surrounding. Label to show all the above points and the regions of the future adult.

(b) The neural folds become higher and approach one another along the dorsal mid-line where they finally fuse. Examine a slightly later stage in which the embryo has become flattened and more elongated and rests on one side. Where do the folds come together last? Where is the blastopore?

**Exercise 14.** Draw this stage from a side view with ventral surface below and label thoroughly as in last.

(c) Understand from lectures and text-book what develops from that part of the ectoderm which is folded in when the neural folds meet and fuse, also the origin of the **neurenteric canal** which for some time connects the neural tube and the archenteron, also the structures which would appear in sagittal and transverse sections of such a stage.

### BEGINNING OF THE TADPOLE STAGE

(a) After the foregoing stage the embryo becomes more elongated and assumes the shape of a crescent. Study such a

stage to identify the dorsal mid-line where the neural tube has disappeared beneath the surface and the anterior end where there is more suggestion of differentiation than at the posterior extremity. The larva can be propped up against a bent pin or some slightly flattened shot and the **proctodaeum** found as a minute pit in the posterior region of the ventral side. Along on each side and just below the dorsal mid-line are some transverse markings which show the lines of division between the **mesoblastic somites,** or first muscle segments. Identify **head, neck** and **body regions.** As not all the larvae given out will be in exactly the same stage, go over this last with one of the instructors to make sure you understand what can be recognized in the particular specimen you have.

**Exercise 15.** Draw a side view showing all these points and having the orientation the same as in your last figure of the neural fold stage, i. e., ventral surface below and head pointing in same direction.

(b) Get several specimens showing the transition from the above to the next stage. Note how the crescent straightens out and the elongated larva gradually shows more **definite** rudiments of the adult organs. In the stage to be drawn, examine from the side, then prop up against a bent pin and study the ventral surface. The **eyes** are swellings right and left on the head, just below them are two smaller dark areas, the **nasal pits,** or future nostrils. A depression on the ventral side and posterior to the nasal pits is the **stomodaeum.** Rudiments of the **suckers** and three **external gills** appear laterally in the neck region, behind them is a protuberance, the beginning of the **fore-limb.** Note the **tail** and the **proctodaeum** and the general changes in shape. Go over with an instructor to make sure of each point before drawing.

**Exercise 16.** Draw from a side view, labeling thoroughly and oriented as other side views.

(c) Study next a larva about ready to hatch. Identify all the features noted in the last and see to what extent they have changed. If the living specimen is too active, add a few drops of ether or kill with five per cent formalin. In a specimen

still alive you will be able to see the **heart** beating within the **pericardium,** which lies in the ventral region of the neck. The stomodaeum becomes about this time connected with the front of the archenteron and so forms the **mouth.** The same thing happens to the proctodaeum which then becomes the **anus.** Note the very dark **pigment cells** scattered everywhere and look for the beginning of a collar like ridge on the ventral surface and extending up laterally along where the external gills are attached. This is the **operculum** and if not yet recognizable will be seen in the next stage.

**Exercise 17.** Draw from side view with orientation same as previous figure and label all the structures which can be shown in this view.

## TADPOLE STAGES

(a) Examine larvae, or **tadpoles,** which have been sometime hatched. Watch them alive, notice their active movements and how the suckers are used. In a specimen anaesthetized with a few drops of ether, examine the gills with low power of your microscope. Watch the flow of blood in the capillaries. Note **corpuscles** and the **pulse.**

**Exercise 18.** Draw the outline of a single gill and its vessels and show course of blood flow with arrows.

(b) With the microscope or hand lens note the heart beating in the pericardium. In some specimens, portions of the digestive tract will be seen showing through the ventral part of the body wall. Note again the operculum and recall later when examining the similar structure in the tadpole of a large bull frog. Note the size and outline of the mouth when seen from below.

**Exercise 19.** Draw this larva from a ventral view.

(c) Examine Museum or living specimens of the genera **Amblystoma, Diemyctylus** and **Necturus.** Notice the external gills which persist throughout life in the last. Examine also specimens dissected to show the viscera in place. These forms are **Amphibia** of the class **Urodela** and are characterized by the

persistence of the tail in the adult. How do they differ from the class Anura?

## VI. THE TADPOLE OF A LARGE FROG

(a) The tadpoles or "pollywogs" of the frogs and toads (**Anura** or tailless Amphibia) have slightly different outlines from the Urodele tadpole just studied. In the case of our smaller frogs and of the toads, the tadpole undergoes the metamorphosis into the miniature adult the same season that the egg is laid, but in the case of the large "bull-frog" the eggs are not laid until late in the spring and the embryo remains in the tadpole condition over the next winter, finally undergoing metamorphosis when about one year old, or in some cases remaining a tadpole through a second year. Such specimens approaching metamorphosis are abundant in the early spring and in fact may be obtained at any time during the open months.

(b) Watch the living specimens in an aquarium or large pan of water; notice how they come up to breathe and how the most advanced specimens are beginning to use their legs.

(c) Study a preserved or a freshly killed specimen in a dissecting pan and covered with water. Make out **nostrils, mouth** with horny **teeth, hind limbs, anal opening** and on one side of the neck region an opening which leads into the **gill chamber,** probe gently into this with a guarded bristle and find the extent of the cavity into which it opens.

**Exercise 20.** Draw from a side view orienting same as drawings of the Urodele tadpole.

(d) Pin the specimen out under water ventral side up. Remove the outer covering from the cavity just explored with the bristle. Find the **internal gills** and between them the **gill-slits.** Discover relation of these slits to mouth cavity.

(e) Take a small fish and pin it out beside the tadpole. Note the flap or **operculum** which covers the gills on either side. Cut off these opercula and find out the relation which the gills and gill-slits of the fish have to its mouth cavity.

Compare this with what you see in the tadpole. Understand how the membrane covering the tadpole's gills is homologous to the fish's operculum. See models showing development of operculum. Understand difference between external and internal gills.

(f) Find the **heart** on mid-line between gills in both tadpole and fish. What parts can you recognize in each?

(g) Remove the ventral body wall of the tadpole from the region between heart and anus with care not to injure the viscera. Identify, by pressing aside without cutting or tearing, the **stomach,** the tri-lobed **liver,** the **gall bladder, pancreas, fat-bodies,** the much coiled **intestine** and the posterior end of **kidneys.** Compare with same in adult frog, as previously studied.

(h) Cut open the abdominal cavity of the fish and examine its viscera briefly for comparison.

**Exercise 21.** Draw the organs of the tadpole as they appear from ventral view and lying in place. Scale of 3. Make outline of body around them.

(i) In the tadpole find the **lungs** and the **oesophagus** where it enters the abdominal cavity. Cut this last off and without injuring the lungs, kidneys, and fat-bodies, remove the digestive tract severing it again at the rectum. Unravel the intestine, spread out and measure using the distance from mouth to anus as a unit. Record and compare with your record of same in the adult frog. Examine now the organs which remain in the body cavity, **lungs, kidneys, spleen,** rudiments of **ovaries** or **testes** and the **fat-bodies.**

**Exercise 22.** Draw on same scale as last to show these organs in place.

(j) With a sharp scalpel, cut through the body transversely, just back of eyes and again a little farther back. Study such a section under water, noting position and development of portions of central nervous system thus exposed and whether any of the bony skeleton is present.

**Exercise 23.** Draw such a section.

## VII. TADPOLES IN METAMORPHOSIS

(a) The miniature bull-frogs in which the tail is still present and which are just completing the metamorphosis, are not as readily obtainable in large numbers as the similar stage in the smaller frogs which metamorphose during their first summer. Examine museum or living specimens of the larger form and then study in a watch glass covered with water a similar stage of one of the smaller species. Note whether the shape of head and mouth have changed and if the **tympanum** has yet appeared; also the development of the **limbs** and **tail,** which last, from this stage on, rapidly dwindles away.

**Exercise 24.** Draw on a scale of 2 or 3 from a dorsal view.

## EMBRYOLOGY OF THE CHICK

(a) Examine the reproductive organs of a male and those of a laying female to see the **testes** and their **vasa deferentia,** the **oviduct** with its **funnel,** its **albumen** and **shell-secreting** parts and its relation to the **cloaca** and **rectum.** Notice in the **ovary** the **eggs** in various stages and the places where eggs have been recently discharged, also the **stigmata** or non-vascular areas which rupture when an egg is set free.

(b) Examine under the microscope a small amount of the **yolk** obtained from one of these ovarian eggs. Recall the similar structures in the frog's egg.

(c) Examine demonstration sections showing the cellular nature of the ovary.

(d) Take an **unincubated** hen's egg and, using scissors, cut open on one side a space about one inch across, being careful that the scissors points do not cut too deep and injure the yolk. The opening may be further enlarged, if necessary, the egg resting upon a bed of cotton wool in a finger bowl. Find the **chalazae** or twisted cords of albumen at either end. What relation have they to the yolk and to the shell? Find the two **mem-**

**branes** which line the shell. These can always be seen at the large end where there is a space between them. At one place upon the surface of the yolk is a small whitish area, the **blastoderm,** the central part of which is known as the **area pellucida** and the peripheral part as the **area opaqua.** Does this always appear at the top, however the egg is turned? Compare with the rotation of the frog's egg in its capsule. Understand the comparison between such an egg as this and that of the frog and the starfish and the condition of the blastoderm at this stage.

**Exercise 1.** Draw the egg thus dissected, on a scale of 1.

(e) Open an egg which has been under incubation for **twenty-four hours** and placing it on the cotton beside the one just drawn compare the two. Record or make a simple sketch to show the changes which have taken place in the blastoderm during this first day of incubation. Before discarding this specimen, the existence of a delicate **yolk membrane** should be demonstrated by puncturing.

(f) Permanently mounted specimens of the blastoderm and the developing embryo will be issued for the study of approximately the 24, 32, and 45 hour stages, in their finer details. These should be handled with great care lest they be crushed by wiping or by the microscope objectives. These slides are secured by removing the blastoderms, which are then fixed, stained and mounted in balsam. The first to be studied is the **24 hour stage,** in which the following parts are to be made out with the low power of the compound microscope; **neural folds, head folds, mesoblastic somites, primitive streak, area pellucida,** the **vascular area** and the **vitelline area.** Focus carefully to determine the vertical dimension of the parts and compare your results with what is shown by models.

**Exercise 2.** Draw this stage as a full page figure, including a small margin from the vitelline area.

(g) Open a **36 hour egg,** just from the incubator and notice the further changes. With the aid of an instructor, inject some India ink into the cavity beneath the blastoderm and harden the embryo by dropping strong alcohol upon the out-

side. Compare part by part with a permanently mounted specimen of the same stage, placing the latter across the top of a watch glass and against a white background. Study with lens to locate **the parts observed in the twenty-four hour stage.** After identifying these with the hand lens in both the fresh and the preserved specimens, study the mounted specimen further under the compound microscope and make out, in addition to the features seen in the last, the beginning of the **brain vesicles, the amnion, heart, notochord** and any changes in the size and proportions of parts. Here again careful focusing and the comparison of what you see with the models is necessary for the proper understanding of the third dimension.

**Exercise 3.** Draw this stage in a figure similar to the last.

(h) Examine next a freshly opened embryo of **45 to 48 hours** incubation, comparing it with the last. Note the **blood vessels,** the **pulsations of the heart** (which should be counted for the number per minute) and the extent to which the vitelline area has extended over the egg and then treat with India ink and alcohol as before. Study together the specimen thus freshly prepared and a stained and a mounted specimen of the same stage. Find all the structures observed in the thirty-two hour stage and in addition note the **cranial flexure** and the **torsion** of the cephalic end of the embryo, the **fore, mid,** and **hind-brain vesicles,** the **optic vesicles** and the **lens** of the eye, the **auditory vesicles,** the tubular **heart,** now bent into an "S" shape, the **vitelline arteries** and **veins** and the **sinus terminalis,** the **gill bars** and **slits** and the extent to which the **amnion** has developed.

**Exercise 4.** Draw this stage, in a figure similar to the last.

(i) Understand from demonstrations of the later stages of chick and mammal and from the lectures, charts and text-books, how the embryo is related in its several stages to the yolk mass and the significance of the **amnion, allantois** and **yolk-sac** in both mammals and birds.

## THE INSECTS

PHYLUM, ARTHROPODA. CLASS, INSECTA OR HEXAPODA

### I. AN ORTHOPTEROUS INSECT

(a) The grasshoppers and locusts are the most common representatives of this order Orthoptera, and any large specimen of several species, which are common locally, may be used. Living individuals should be observed in glass jars, containing grass and covered with a screen. Exactly how are the legs used in walking and jumping? The **spiracles,** or respiratory openings will be seen along the sides of the abdomen. Observe and time the intervals between the respiratory movements. Note the nature and the distribution of color upon the animal. Can you suggest any value which this may have for the animal in nature? Offer bits of green vegetation to the specimens in the jars and see what you can make out regarding their mode of feeding. Touch the "feelers," **antennae,** of the head with a long piece of glass tubing having a plug of absorbent cotton in the end and observe how sensitive to touch are these organs as compared with other parts of the body. Moisten the absorbent cotton with some strong smelling fluid and bring it near the antennae without touching them. Can the animal smell with these organs or with any other part of the body? Remove a specimen from the jar and holding gently examine the parts at closer range. Look at the **compound eyes, the antennae,** etc., with the lens. Note the "molasses" which is regurgitated from the mouth. This is a digestive fluid mingled with food. If a good sized drop can be collected from one or more specimens and placed upon a slide, put a bit of fresh green vegetation in this and note result before the fluid evaporates. What may be the significance of this habit of regurgitating the contents of the digestive tract? If you have time, devise experiments to determine whether temperature, or sensations

akin to fear in the higher animals influence the rate of the respiratory movements.

**Exercise 1.** Write out in the form of carefully worded notes such facts of the above study as can be thus recorded.

(b) A preserved or a freshly killed specimen may now be used for a study of the structure. Compare the **segmentation** with what you know of the crayfish and earthworm. The main divisions of the body are **head, thorax** and **abdomen.** The thorax has three segments, **prothorax, mesothorax** and **metathorax** and has the three pairs of legs. How many segments in the abdomen? Are there signs of segmentation in the head? The **antennae** and **compound eyes** have already been located, examine again with the lens. Find the **simple eyes,** or **ocelli,** three small dots between the antennae. About the **mouth** are the following parts: the upper lip or **labrum,** next a pair of **mandibles** and, by pressing these last aside, the pair of **maxillae** and the **labium,** which last functions as a lower lip and is really a second pair of maxillae united. Note the labial and maxillary **palps,** or "feelers." The forward, upper and lateral parts of the head are covered by a simple plate of the skeleton, the **epicranium,** with which the mouth parts just mentioned are articulated. Like the exoskeleton of the crayfish, the covering of the grasshopper's body is a continuous membrane which thins out at the joints and is of different thickness in different parts of the body. For convenience we speak of the **plates** or **sclerites, of the skeleton** though neighboring plates are continuous by the thinner skeleton over the joints.

(c) Examine the thoracic region and determine accurately the number and position of the plates in each segment, the place of attachment of the **wings** proper and of the **wing-covers.** Each of the thoracic legs consists of five main divisions: the **coxa** by which the leg articulates with the body, a short segment the **trochanter,** a long segment the **femur,** then the **tibia** and last the three jointed **tarsus** terminating in a pair of **hooks** and a little **pad.** Notice how the trochanter of the metathoracic leg has fused with the femur and how this limb is a special adaptation of the same plan which the others present. The

abdominal segments are divided into upper and lower parts by a horizontal band along which are the **spiracles.** How many of these are there and on which segments do they occur? On the first abdominal segment near the spiracles there are oval areas covered by a thin membrane. These are the **auditory organs.** The tip of the abdomen differs in the two sexes. Each sex has the following parts in common: a single terminal **dorsal plate;** right and left below this the paired **podical plates,** between which lies the anus and on the outer face of which are two small projections, the **cerci.** Below the podical plates of the male there is a single large **sub-genital** plate which is replaced in a female by the conspicuous plates of the **ovipositors.** Understand how the latter are opened for the extrusion of the egg after digging the cavity in which it is laid.

**Exercise 2.** Draw a full page figure showing the grasshopper as seen from the right side. Spread out dorsally the wing and wing-cover of this side and arrange the legs so that the body will show to advantage.

(d) Holding the specimen in one hand cut open the body along the mid-line a little to one side of the mid-dorsal region. Use fine scissors and be very careful not to cut deep and injure the soft parts underlying the skeleton. After opening for the entire length of the body, pin out under water in a dissecting pan by slanting two pins across the union of the head and prothorax. Fasten the free end of the abdomen with a single pin passed through the end of the cut and then spread out and pin apart the edges of the cut, using great care not to injure the internal organs. If the **heart** has not been destroyed in opening the specimen, it will be found either adhering to the inner face of the skeleton, along the dorsal mid-line of the abdomen, or in this position upon the surface of the internal organs. A lace-like mass of yellow tissue is the **fat-body** which should be removed carefully to expose the **alimentary** canal a large tube running straight through the body. In exposing this more fully, the **muscles** of the thoracic region must be removed. On top of the tract in the abdominal region are the

**reproductive** organs which have right and left parts coming up from either side and meeting dorsally. Their ducts pass backward from the ventral side and unite in a single duct which opens ventral to the anus, on the upper face of the sub-genital plate in males, between the halves of the ovipositors in females. Among the organs are certain structures which are conspicuous in the fresh specimen by their silvery color. These are the **tracheal** or **air-tubes** and the **air-sacs** through which the air taken in by the spiracles is carried to all parts of the body. Remove the parts of the fat-body that may conceal the **digestive tract.** Beginning anteriorly, this is composed of a **crop,** which is thin walled; a **stomach,** encircled by eight elongated pouches, the **gastric caeca;** a region called the **intestine** and a terminal portion, the **rectum.** Where the stomach passes into the intestine there will be found a mass of threads which have an excretory function and are known as the **Malpighian tubules.**

**Exercise 3.** Draw the digestive tract on a scale of 3 or 4, as it lies in place and seen from the dorsal side, making around this a simple outline of the body as cut open.

(e) Remove the digestive tract by severing the rectum near its posterior end and pulling gently forward. The short **oesophagus,** by which the crop connects with the mouth, will now be seen. Before this is severed find the brain just back of the eyes and dorsal to the tract, and the **circum-oesophageal** connectives which pass on either side of the oesophagus and connect the brain with the **sub-oesophageal ganglion** (to be seen later). Cut the oesophagus, without injuring any of these parts, and examine the gut under water, to get a better view of its parts; particularly the gastric caeca and Malpighian tubules. Look on the sides of the crop near its posterior end for the **gastric ganglia,** white spots from which fine **nerves** radiate. Look on either side of the body in the region from which the crop has been removed for the **salivary glands.** These communicate by fine ducts with the region just inside the mouth, a point which is difficult to ascertain without a special dissection.

(f) Look at the **air sacs** as now exposed and make out any regularity in their arrangement. Remove a small bit of the

muscle or fat-body and examine on slide under a cover with microscope. Find the **tracheae** lined with their **spiral threads.** Draw if you have time. Continue to remove the fat-body and muscles of the ventral side, in the region of the thorax, until the **nerve cord** is exposed. If the reproductive organs have not been destroyed the union of their right and left parts may be observed below the gut before their removal. How many **ganglia** are there and how many nerves does each give off? How does the position of the entire nervous system in the body compare with the same in a frog, a crayfish and an earthworm? From the **brain** there are **nerves** to the compound eyes, the ocelli and the antennae. The circum-oesophageal connectives have already been located.

**Exercise 4.** Draw as much of the nervous system as you have made out upon the same scale as the drawing of digestive tract.

## II. A COLEOPTEROUS INSECT

(a) Any large beetle will do for this study, provided it is not too highly modified. By examining the animal from the ventral side locate the **head, thorax** and **abdomen** and the number of segments visible in each. Look for **antennae, compound eyes, ocelli, mandibles** and other **mouth parts,** the **anus** and the **thoracic legs,** and compare with what you have found in the grasshopper. Where are the **wing covers** and the **wings?** When the latter are found see how they fold up beneath their covers. Fasten down, dorsal side up, by pinning through the prothoracic segment, spread one wing cover out at right angles and unfold the corresponding wing which can be spread in the angle between the wing cover and **abdomen.** Raise the head, if it bends too far ventrally, and spread out the thoracic legs on the side where wing and cover are closed.

**Exercise 1.** Draw the specimen from this view and on such a scale as to make the figure three or four inches long. Show the plates of the skeleton with care and number the segments of thorax and abdomen.

(b) In the larva of a beetle find the main divisions of the body, **head, thorax** and **abdomen, mouth** with its **jaws** and the **anus.** Count the number of segments comparing with adult of the same species.

**Exercise 2.** Draw such a larva from a lateral view showing these parts on a scale of 3 or 4.

(c) Examine, as directed by instructor, such living specimens of beetles and their larvae as are available for individual study or demonstration.

## III. A HYMENOPTEROUS INSECT

(a) Wasps of the genus **Polistes** are very common and are easily collected when they enter unscreened buildings with the approach of cooler weather in the fall. **Head, thorax** and **abdomen** will again be recognized as in the case of the other insects. How many segments in each? Look for **antennae, compound eyes, ocelli, mouth parts** and **anus.** At the posterior end of the female is the **sting.** The **spiracles** are a row of minute dots on each side of the abdomen. Compare the divisions of the thorax and of each **thoracic leg** with the corresponding parts of the grasshopper. To which segments are the **wings** attached?

**Exercise 1.** Draw a side view with wings spread dorsally, on a scale of 3 or 4.

(b) Examine the "paper" nests of this wasp and others if available. Also artificial **ants' nests** and the **eggs** and **larvae** recently taken from an ant colony. The most remarkable facts regarding the hymenoptera are those connected with their social life in such colonies, a matter which will be discussed in lectures or text-book.

## IV. A LEPIDOPTEROUS INSECT

(a) Examine a good sized butterfly, or moth, going over the features noted for other forms (the three main divisions of the body, **eyes, antennae, mouth parts, legs** and **wings**).

Mount some of the dust from the wing surface and examine under a microscope. What is the significance of the term "lepidoptera"?

**Exercise 1.** Draw a dorsal view with wings spread, making the figure three or four inches across. Omit color pattern.

(b) If available the eggs of butterflies or moths will be shown as a demonstration. Understand to what species such eggs belong and where they are laid.

(c) Examine now a larva which is large and favorable for study. Where are the **head, thorax** and **abdomen?** Do you find **thoracic legs?** There are other pairs of appendages somewhat like them and known as **prolegs.** How many are there and what is their structure as compared with the thoracic legs? Are there **eyes, ocelli, antennae** and **mouth parts** as in other forms? Do you find spiracles?

**Exercise 2.** Draw a side view on a large scale.

(d) If it is the proper season the larvae of various forms will be placed in the laboratory for individual study or demonstration. Observe the way of moving and their voracious habits in feeding. How does the structure and use of the mouth parts differ in larva and adult? At the proper season, caterpillars will often spin their cocoons in the cages where they are kept, or such cocoons may be collected and given out for study. Cut one open and find the resting stage, "pupa," within. Notice the silk of which the cocoon is composed. Such cocoons if uninjured may be kept in cages and the emergence of the adult insect observed at some subsequent time.

(e) Understand the complete life history in each of the groups thus far studied and be able to explain the difference between **direct development** as in the grasshopper and the **metamorphosis** of butterflies, beetles, etc.

## V. OTHER ORDERS

(a) Of the remaining orders of the Insecta, three are more commonly known and recognized by popular names. These are the **Hemiptera,** or true bugs; the **Diptera** or two-winged

flies, of which the house fly is our most common representative, and the **Odonata** or dragon flies. Representatives of these and of their larvae will be placed in the laboratory for demonstration and supplied to individuals if called for.

(b) The life history and habits of insects presents much which is even more profitable for study than the points heretofore covered, but such work is difficult to handle properly with large classes and at fixed periods. Suggestions and assignments will, however, be made upon request to the instructor in charge and facilities for carrying on such work either at home or in the laboratory will be provided.

## PARASITISM AND OTHER FORMS OF ASSOCIATION AMONG ANIMALS

### I. A PLANARIAN WORM

Phylum, Platoda. Class, Turbellaria

Planarian worms are common in fresh-water where they are most easily discovered on the under sides of leaves, stones and small objects upon the bottom. For this study, the species **Planaria maculata** or any other representative form of these turbellaria may be used.

(a) Examine in a watch-glass of water. How does the animal move? What changes in shape may the body undergo in "righting"? In meeting obstacles? In response to other stimuli? What is the shape and distinctive feature of the two ends? Are there sense organs? Can you find the **mouth** and, in sexually mature animals, the **genital aperture** on the ventral side? Transparent specimens will show the dendritic branches of the **digestive system,** the plan of which should be understood from chart or text-book figures. Place a small specimen upon a slide under a cover-slip and look for **cilia.**

Can you see the justification for applying the name turbellaria to these forms? Study also the coloration under microscope and hand lens.

**Exercise 1.** Draw the animal from a dorsal view, scale of 8 to 10, showing the above features.

(b) Specimens will sometimes feed if crushed snails or bits of meat are placed in the dish. The muscular **pharynx** may then be seen. In this connection, their actions may be watched for evidence of a **sense of smell.** Interesting observations may also be made upon **regeneration** and upon their behavior with respect to light. Carry out such observations and experiments if you have time.

(c) These worms are studied here with a view to emphasizing their **free-living** condition. In nature, they move about actively, often capturing living prey, and reproduce either vegetatively by **fission** or by means of **germ-cells.** They are hermaphroditic. The eggs are laid in small stalked capsules attached to the under sides of the stones and other objects upon which the animals are living. **From each of these capsules or egg-shells a number of young emerge as miniature adults able at once to take up the life of the parent upon the bottom.** The life-cycle is thus in marked contrast with that of the parasitic representatives of this phylum.

## II. A FLUKE-WORM

### Phylum, Platoda. Class, Trematoda

To the **trematoda** belong the external and internal parasites known as the **fluke-worms.** For this study, any one of the genera somewhat resembling **Distomum** may be used. **Haematoloechus variegatus,** from the lungs of the frog, and another genus often found free in the body cavity of the frog are excellent material.

(a) Examine living or preserved specimens and locate the **mouth** and **suckers.** How does the shape and behavior, if specimens are observed alive, compare with the same in the

planarian? Locate the **digestive tract** and compare with the planarian. The **reproductive organs** are complex and varied in appearance and, if studied, special directions will be given. The animals are **hermaphroditic** and produce fertilized eggs which accumulate before being laid in a terminal portion of the female organs, the **uterus,** developing later when they are laid. The life-cycle is greatly complicated by the parasitism as described in lectures or text-book. Both in the structure and conditions of the life-cycle, contrasts should be drawn between the trematode and the planarian.

## III. A TAPE-WORM

### Phylum, Platoda. Class, Cestoda

The **cestoda** or tape-worms are parasitic forms, even more highly modified in correlation with their parasitic habits than are the fluke-worms. The adults occur as parasites within the digestive tract of another animal, the larval stages mostly within the tissues of a **secondary host** upon which the **primary host** is likely to feed. Species of the genus **Taenia** occur in many common mammals and are excellent for the study of the external features. They may be examined alive in water or after preservation in alcohol or formalin.

(a) Examine an adult cestode in a pan of water. The smaller end has a head, **scolex,** the posterior end ripe joints or **proglottids.** What structures adapted for holding fast are found upon the scolex? Count the proglottids, compare with numbers in neighboring specimens and record. Can you see indications of the developing reproductive organs and of the **genital apertures** with **copulatory organs,** at one side? How and where do the proglottids seem to originate? **Each proglottid contains a complete hermaphroditic reproductive system** and the chain of proglottids may be regarded as a reduplication many times over of the reproductive machinery. If living worms are available, test the firmness of their attachment by the scolex to the mucus membrane of the host. Com-

pare the external features with those of other tape worms shown as museum specimens. **Moniezia expansa** from the sheep and **Crossobothrium laciniatum** from the sand-shark are good for this purpose.

**Exercise 1.** Draw a good sized figure of the adult, indicating the parts as above and with the proglottids accurately shown. To avoid repetition, the figure may show several representative regions of the worm connected by dotted outlines.

(b) The "ripe" proglottids at the posterior end often show the outlines of the distended **uterus,** a cavity in which the development is begun. Here, the egg develops as far as the **six-hooked** embryo, a stage which may be obtained from either living proglottids or formalin material. Cut the proglottid into bits in a watch-glass and examine some of the material under high power of the microscope. Embryos surrounded by a tough **shell** and other membranes will be found. Can you find the six **hooks?** Is there any remnant of the **yolk** material? If alive, crush by pressing on the cover and watch movements. Do they seem effective as "boring movements?" Can you make any estimate of the number of six-hooked embryos produced by a single cestode?

**Exercise 2.** Draw the embryo and its surrounding parts.

(c) The ripe proglottids, with their six-hooked embryos, break off and pass out with the feces of the host. The six-hooked embryos are discharged by the rupture or disintegration of the proglottid and find their way to the secondary host by the chances of nature, entering with the food or drink. After its membranes are digested in the stomach of this host, the six-hooked embryo bores out into the tissue and develops to a stage known as the bladder-worm. Examine living or preserved material and make out the **scolex** and **neck** and their peculiar position. Can you see anything adaptive in this development within the bladder? Understand what happens when the bladder-worm is eaten by the primary host.

**Exercise 3.** Draw the bladder-worm, making a good sized figure.

(d) If the internal structure of the proglottid is to be studied, excellent results may be obtained from the motile proglottids of **Crossobothrium laciniatum** stained with alum cochineal. Further explanations will be given in the laboratory.

(e) Other important features in the internal structure of the cestode, which may be noted in chart or text-book figures or a further laboratory study, are, the **brain** and **nerve cords, excretory system, absence of a gut,** the granules of $CaCO_3$, and the **cuticular membrane** which covers the body. Are any of these features to be correlated with the parasitism? How may cestodes and trematodes be homologized?

(f) All tapeworms, so far as known, have two hosts. In the following cases of known life-cycles which would be the primary and which the secondary host? Man,—pig; dog,—rabbit; mouse,—cat; house-fly,—hen; fish,—bird; louse,—dog; man,—fish. Consider where and how each of the three stages would occur in each case.

## IV. OTHER FORMS OF ASSOCIATION AMONG ANIMALS

The platoda offer an excellent example of closely related animals existing as free living and as parasitic organisms. From what you know regarding these and any other parasitic forms, write out a statement of the kind of modifications in structure and life history likely to occur in animals which have assumed parasitic habits. Consider cases of **communal parasitism** in insects as explained elsewhere and the striking cases of association occurring among marine animals such as crabs, worms and mollusca, and the whole in relation to lecture, text-book and field-work bearing upon the inter-relations among different species and the struggle for life.

## THE CLASSIFICATION OF ANIMALS

### I. THE PHYLA OF THE ANIMAL KINGDOM

A sufficient number of forms have now been studied in detail to enable you to form some picture of the types of structure which existing animals present. These may be reviewed and your knowledge extended in certain places with a view to giving you concrete ideas of other types not yet examined. The term **Phylum** is applied to certain larger groupings of animals though, as we shall see at the conclusion of this survey, the phyla may again be grouped into larger subdivisions. An examination of the several phyla by means of museum or other specimens may be accomplished as outlined below.

**Phylum, Protozoa.**

The forms studied, **Amoeba, Paramoecium, Englena,** and **Gregarina,** together with figures and demonstrations of other forms, sufficiently illustrate this phylum. Emphasis is centered upon the unicellar state, though simple colonies frequently occur.

**Phylum, Porifera.**

This phylum comprises the sponges. Examine simple sponges like **grantia** and **leucosolenia,** and notice the **osculum,** an excurrent opening, and, in grantia, the many small **pores** by which the water passes in. The larger sponges like the **bath-sponge,** and others which may be upon exhibition, are regarded as derivatives of the simpler types modified along the line of extensive **budding** and **vegetative growth.** Characteristics of the sponges are: Absence of a stomach or gut cavity comparable to that of other many-celled forms; attachment except during the earliest stages of development; absence of what may be properly termed organs, although there are different kinds of cells; a skeleton of fibres as in the bath sponges

or spicules as in grantia or the "glass sponges." The sponges are clearly the simplest of the many celled animals now living.

**Phylum, Coelenterata.**

**Hydra** and the **hydroids** with their **jelly fish** are the examples studied. Examine other and larger **jelly fish,** a **sea anemone, corals** showing the soft parts and the skeletons, **sea-pens, fans,** etc., and chart figures of **ctenophores.** Distinctive features are: absence of an anal opening; radial symmetry; two layered or diploblastic structure, i. e., ectoderm and endoderm as in hydra; tentacles surrounding mouth and in most cases attachment during a part of the life-cycle.

**Phylum, Platoda.**

**Planarians** and similar forms, **fluke-worms** and **tape-worms** as studied. Characterized in the forms not degenerate through parasitism by: bilateral symmetry; absence of an anal opening; absence of a coelomic cavity, though there is a middle germ layer (mesoderm) and the animal is therefore triploblastic.

**Phylum, Annulata.**

The **earthworm** is a representative form, though the group as a whole is characterized by appendages on the segments and organs of special sense in the head region which the earthworm has presumably lost in the course of its evolution.

**Nereis** is in this respect a better example. Small species, related to the earthworms, are common in fresh-water and may have been observed. There are many **tube-building forms** among the marine worms, museum or chart figures of which may be examined. The **leeches** of fresh-water are greatly modified annulates. The annulata are characterized by: bilateral symmetry; an obvious metamerism; three germ-layers; a well developed coelome; nephridia; dorsal brain and ventral nerve cord as in earthworm.

**Phylum, Mollusca.**

Represented by the **clams, mussels, snails, slugs,** etc. Examine specimens of bivalve shells, comparing with the fresh-

water mussel, also the shells of marine snails showing lines of growth and peculiar shapes. Watch living **pond snails,** if not already studied, and notice the symmetrical head and creeping foot. Examine museum specimens of the **limpets,** marine forms belonging with the snails and adapted for clinging tightly to the rocks with the foot. Also another type of mollusc the **chiton,** with its segmented shell. Examine another type represented by the **squids, devil-fishes** and **cuttle fish,** active, free, swimming forms with an internal shell. As a phylum, the mollusca are characterized by: bilateral symmetry; a ventral foot; a dorsal one-pieced shell; a mantle, enclosing a mantle cavity in which lie gills; absence of metamerism; presence of a coelome with nephridia; three germ-layers. Most of these characters may be recognized in the specimens placed upon exhibition.

**Phylum, Echinodermata.**

Examine specimens of **starfish** and **sea-urchins,** noting the **five-rayed symmetry.** Can you find mouth and anus? Organs known as the **tube-feet** are used for locomotion. See macerated specimens showing **plates** of skeleton. Examine **sea-cucumbers** and compare, noting the difference in the relation between body axis in relation to normal position. Can you see the five rays? Tube feet? How would you homologize the outer surface of a sea-cucumber and a sea urchin? The sea cucumber and a starfish? The echinoderms are a very aberrant group. For a long time classed with the coelenterata because of their radial structure, they have since been clearly shown to be forms with far greater complexity of organization and are clearly animals which have been modified from bilaterally symmetrical ancestors possessing three germ-layers and a coelome. See demonstrations of the **bilateral larval forms.** Distinctive characters of the phylum are: a radial symmetry, masking a more fundamental bilateral symmetry, which appears in the larva; an extensive coelome; a water vascular system used for locomotion and unique in the animal kingdom; a skeleton consisting of isolated plates em-

bedded in the mesoderm; a simple type of nervous system and sense organs.

**Phylum, Arthropoda.**

To this phylum belong the familiar **crayfish, crabs, insects, spiders, scorpions,** etc. Examine museum specimens of crayfish and lobsters, if not already dissected, and compare with a crab, noting, **mouth** and **anus, eyes, antennae,** jointed **appendages,** external evidences of **metamerism.** In all these forms the relation of the nervous system to the gut is similar to that in the earthworm or crayfish. Examine **water-fleas,** as they swim in an aquarium and in a watch-glass after anaesthetizing with ether. In what respects do they agree with the larger forms in structure? Characteristic features of the phylum are: a continuous cuticular skeleton, thinner at the joints; bilateral symmetry; metamerism; a pair of jointed appendages on each segment; a pair of compound eyes; three germ layers. A well developed coelome is not present but it seems probable that such was the case in the ancestors of this group.

**Phylum, Chordata.**

The vertebrates constitute by far the most conspicuous members of this phylum and are sufficiently familiar without further examination in this connection. Other less known forms are the **amphioxus** and the **tunicates** or **sea squirts.** Examine specimens. The amphioxus with its elongated **metameric body, mouth** and **anal** openings and **fins** bears a remote resemblance to a fish. It can, at least, be imagined when viewed from the outside to have some resemblance to the simpler vertebrates. Internally there are **gill-slits,** a **notochord,** a **dorsal tubular nervous system** and other vertebrate characteristics. The sea-squirt, on the other hand, would never suggest such a relationship. It is adapted for a sessile life and possesses an inhalent and exhalent siphon much like those of a clam. Indeed these animals were for a long time classed with the mollusca. Later, it was shown that the embryo possessed the notochord, dorsal tubular nervous sys-

tem and other features found in no other group but the vertebrates. Accordingly the tunicates were placed in a group with the vertebrates. Distinctive features of the chordata are: a dorsal tubular nervous system, formed by infolding as in the frog embryo; a notochord, present in embryo only (frog) or throughout life (amphioxus); metamerism more or less conspicuous; three germ layers; coelome; bilateral symmetry, etc.

## II. PHYLOGENY OF THE ANIMAL KINGDOM

(a) After this review of representative types we may next consider the grouping of the phyla into larger divisions. Here we must consider only a few very fundamental points of structure such as, the many celled or single celled condition; with a gut cavity or without a gut cavity; diploblastic or triploblastic; with a coelome or without a coelome; with metamerism or without metamerism.

**Exercise 1.** Construct a table showing how the phyla will group themselves when classified along broad lines.

(b) Looked at in another way, this table will represent a family tree of the animal kingdom for it probably gives us the broad lines along which evolution has proceeded. Do you understand now the meaning of a **natural classification** or one based upon blood relationship and the meaning of structural resemblance among animals?

# APPENDIX

## A BONY FISH

Any of the common bony fish will serve for this study. The marine Ctenolabrus, fresh-water perch, or any of the minnows. They should be collected during the summer and preserved in formalin. Interest in the work is greatly increased by having a few live fish to study action of fins and manner of respiration.

(a) **External Characters.** What is the shape of the body as a whole? How is it adapted for motion through the water? How do you distinguish anterior and posterior ends? Dorsal and ventral surface? Note the **head, trunk** and **tail** regions. Is there a neck? Are these regions sharply marked? What is the nature of the coverings of the body? 1. **Head:** Its shape? **Mouth, nostrils, eyes, gill-openings, operculum,** which consists of several parts. Beneath the operculum is the **branchiostigal membrane,** supported by bony rays. The branchiostigal region is connected with the trunk by the narrow isthmus. Its shape? Relation to gill openings?

(b) **Trunk.** Its general shape? How does this region compare as to length, height and thickness? Describe the color of your specimen? Compare several specimens, and male and female. Describe the variations noted. Note the lateral line on each side of the body. Can you trace it on the head? Determine what causes the line.

(c) **Fins.** How many? How many in pairs? Single? What ones are in the median line of the body? How are they supported? The fin on the back is the **dorsal.** Are there one or two? Compare as to size and supporting rays. The terminal fin is the **caudal,** and the one just behind the vent is the **anal.** Compare each with the dorsal. Do the paired fins have supports? What is their position on the trunk? The

anterior pair are the **pectorals,** the posterior the **pelvic.** Compare several kinds of fish and determine the variation in position of these fins. If practicable, the living fish should be studied in aquaria to determine the use of each fin.

(d) **Integument.** Describe the distribution and arrangement of the scales. Is it regular? Do they extend into the head? Is there skin over the scales? Is the pigment in the scales? Pull one out. The rounded marginal scales are **cycloid,** the toothed or spiny marginal are the **ctenoid.** Sketch.

(e) **External Apertures.** Determine the position, size and shape of the following: (a) **mouth,** (b) **vent,** (c) **urino-genital,** (d) **nostrils,** (e) **gill openings.**

(f) **Mouth Cavity.** Its general shape? Are there lips? The bony framework consists of the upper jaw, a **pre-maxillary** in front and a **maxillary** behind. Shape and size of each? The lower jaw is the **dentary.** Which ones bear teeth? Are there other bones that have teeth? Where? How many rows of teeth in jaw bones? Their shape and size? 1. **Tongue:** Shape? Attachment? Size? 2. **Oesophagus:** Position? 3. **Gills:** Lift operculum and examine the gills. Each is composed of a bony arch and numerous gill filaments. On the inner edge of the bony arch are a number of short processes, the gill-rakers. How many gill-slits? Their relation to the pharynx? Are all the gills alike? Compare first and second and the last two. What use can you suggest for the gill-rakers?

**Exercise 1.** Draw as seen from one side, showing the mouth and gill region dissected.

## A POND SNAIL

For the following study the French snail **Helix** will be found very satisfactory, but the common pond snails **Physa, Planorbis** or **Limnea** may be used to advantage.

(a) **The Shell.** Is there any division into valves? What is its general form? How many turns does the shell make? How do they vary in size? Compare several specimens of the same species as well as several different species. Do the coils turn to the right, **dextral,** or to the left, **sinistral?** Is the coiling loose or close, flat or conical? The **apex** of the shell is the oldest part and corresponds to the umbo in the clam. The wide opening is the mouth or peristome (of the shell not the animal). What is its shape? Is the margin smooth or toothed? Explain. One side of the peristome is in some species drawn out into a spout-like process. What is its use? Do you find in any of the species an oval plate closing the opening? In some snails there is such a structure which is called the **operculum.** To what is it attached? Can you explain its use? The whorls make a line where they come in contact; this is the **suture.** Is the suture a simple or an irregular line? Explain the lines that run parallel to the edge of the peristome as **lines of growth.** Is there any variation? Explain. The whorls coil around a central axis, the **columnella.** Describe it in a demonstration specimen of some large shell, making a sketch to show its parts.

(b) **The Living Animal.** How does the snail move? What is the shape of its foot? Its relation to the rest of the body? To the shell? The anterior region of the foot is termed the **propodium;** the posterior the **metapodium;** and between these two is the **mesopodium.** Are these regions sharply marked off? To which part is the operculum attached when present?

(c) **The Head Region.** Dorsal to the foot find the **mouth.** Shape? On each side are fleshy tentacles. Their size and shape? Touch with needle. What happens? Are there one

or two pairs? Do you find small glistening spots, the **eyes,** on the tentacles? Their position? Locate the **anal opening** on the right side of the head. In the air breathing snails find the respiratory opening near the anal opening.

**Exercise 1.** Draw from a side view, showing foot, head and shell as they appear when fully expanded.

(d) The snail belongs to one of the sub-divisions of the **Mollusca** known as the **Gastropoda.** Explain the application of this term. From your study of the clam and snail, write a definition of the group Mollusca and the sub-group Gastropoda.